

THE USE AND ABUSE OF THE DEGREE DAY CONCEPT IN FORENSIC
ENTOMOLOGY: EVALUATION OF *COCHLIOMYIA MACELLARIA* (FABRICIUS)
(DIPTERA: CALLIPHORIDAE) DEVELOPMENT DATASETS

A Thesis

by

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ABSTRACT

The Degree Day method allows the temperature-dependent growth of poikilothermic organisms to be measured within their upper and lower thermal limits. It has been used in agricultural and entomological sciences for more than a century. Subsequently, it has been adopted, by means of the Accumulated Degree Hour (ADH) or Accumulated Degree Day (ADD) by the forensic entomology community as a way of predicting the growth of arthropods, most commonly in homicide cases. However, despite being used in casework, development data created using this model have rarely if ever been validated at the time of their making nor have they been subject to field evaluation using human remains. Forensic sciences have come under considerable scrutiny by the legal system in recent years and as such it is necessary to create more robust data and modeling systems for evidential analysis. The current study took samples at four different time points from 29 sets of human remains. These samples were used to evaluate the accuracy of two development datasets pertaining to the blow fly *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) to predict the actual time of placement (TOP) of each set of remains. The phenotypes, stage (time to third instar), length and weight were used to predict TOP in each case and although stage performed the best overall (29 out of 80 cases), in real terms none of the development datasets performed at a level that might be considered accurate enough for evidential analysis in this study. A discussion of the caveats of the Degree Day model are presented, accompanied by suggestions for improvement in developing future development datasets and a call for better evaluation and validation of forensically-oriented entomological studies.

DEDICATION

I dedicate this thesis to my family and friends in England who have carried me through every part of my time here in the US. I am especially grateful to Robin for his personal sacrifices and emotional support. I love all of you.

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The computer application (ADH monitor) for Chapter Two was developed by Dr Robin Fencott.

Most of the larval samples collected from human remains were collected by graduate students at the FARF Facility, Ms. Chloe MacDanel, Ms. Devora Gleiber and Ms. Lauren Meckel.

Plots were developed using code originally written by Dr Meaghan Pimsler using R statistical package (R Foundation for Statistical Computing, Vienna, Austria)

All other work conducted for the thesis was completed by the student independently.

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NOMENCLATURE

ADD	Accumulated Degree Day
ADH	Accumulated Degree Hour
TOP	Time of Placement
TOC	Time of Colonisation
PMI _{min}	Minimum Postmortem Interval
FARF	Forensic Anthropological Research Facility

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1. INTRODUCTION AND LITERATURE REVIEW

“The same grain is harvested in very different climates; it would be interesting to make a comparison of the sum of the temperatures for the months during which the cereals accomplish a greater part of their growth and arrive at a perfect maturity in warm countries. . . temperate countries. . . and in cold countries.” - *Rene Antoine Reaumur*

The understanding of a possible relationship between organismic growth and temperature can be traced back to this quote by French biologist, Reaumur [5]. Although the term ‘sum of temperatures’ appeared to have no real theoretical grounding at the time, Reaumur had hit upon a fundamental facet of how climate and temperature play a role in the growth of much of life on earth. The Mammalia and other homeothermic animals make up only a small proportion of known taxa. In fact, poikilotherms - organisms whose body temperature are regulated by the external environment and are extremely variable - represent almost all species including plants, vertebrates and Arthropoda. This suggests that climatic factors have played an important role in evolutionary history [6]. Around a century following Reaumur’s ideas, the concept of temperature-dependent development began to take shape as scientists and agricultural researchers took an interest in measuring the growth of crops and their pests. With an increasing demand for agricultural output by a growing human population, it became necessary to understand the life histories of crops, to maximise yield and inform growers of optimal annual planting and harvesting dates. Shortly thereafter, insect scientists found the same theory applicable to insect pests and were able to utilise the same models to improve their understanding of insect life histories and population dynamics. As such, the complexities of the relationship between development and temperature became the subject of intense investigation by entomologists and agricultural researchers alike.

More recently, the temperature-dependent relationship of insect growth has been employed by the forensic sciences [1, 7]. Of particular interest are the blow flies (Diptera: Calliphoridae) as these are typically the first to arrive at remains postmortem, making them excellent timepieces for decay. Blow flies are regularly reared in controlled experiments to produce datasets from which degree day models may be produced. Subsequently, these models can be used to make predictions of the minimum postmortem interval (PMI_{min}) of a set of remains. For the purposes of casework, PMI_{min} (e.g., the time of death or placement of remains) is assumed to be the same as Time of Colonisation (TOC) of the remains, given that blow flies are assumed to arrive immediately upon death, sometimes within just 30 minutes. However in reality this may be far from accurate - insects may not have immediate access to remains or they may have infested a body whilst it was still alive, a state known as myiasis [2, 8].

This review will focus on the myriad aspects of development in blow flies, as well as a critique of the current methods utilised to measure them. We will specifically detail the degree day modeling method, its caveats and subsequent impact on the field of forensic entomology. We close with a discussion about the use of the words ‘validation’ and ‘evaluation’ and how their misuse and misunderstanding can be implicated in one of the major issues with forensic entomology and forensic science practice as a whole.

1.1 Forensic entomology

“Contrary to the popular adage, dead [people] do tell tales” - *Ken Schoenly, Lee Goff, Jeff Wells and Wayne Lord*

Forensic entomology, sometimes referred to as medico-legal entomology is, broadly, the utility of arthropods as evidence in legal and criminal proceedings. It has a long and intriguing history, dating back to ancient China and the book "Washing away of wrongs" authored by Sung Tz'u [Figure 1a] [9, 10]. Over the centuries since then, many investiga-

tors, doctors, anatomists and even artists and sculptors have observed and depicted the role of insects, particularly the larvae of blow flies (Diptera: Calliphoridae), in the decomposition of human remains. Moreover, television shows, radio and film have brought forensic entomology into the public eye.

Entomological evidence is legion and may be used in cases of food contamination (stored product pests), abuse and neglect (both human and animal myiasis) and public nuisance or disturbance (urban pests). However forensic entomological evidence is probably most well known for its application to homicide investigations, typically where arthropod samples are recovered from decomposing human remains. This has resulted in the blossoming field of carrion decomposition ecology, where basic research in ecological and biological processes such as succession, ephemeral resource use, microbiology and competitive interactions have informed the understanding of carrion as a discrete ecosystem [2, 11–13]. In the past two decades researchers have uncovered multiple processes determining the decomposition of vertebrate carrion in the presence of insects and arachnids. In fact an entire book is now dedicated to the complex nature of carrion ecology from microbial decomposition to grave-soil chemistry and beyond [14].

Although traditionally informed by models depicting the temperature-dependent nature of carrion-using arthropods, forensic entomology in human remains systems now encompasses a wide variety of fields including quantitative and population genetics [15–17], ecology [11], evolutionary biology [2], microbiology [18] and even computer science [19, 20]. Forensic practitioners use knowledge of carrion-using arthropoda, their predictable patterns of succession on vertebrate carrion and development times to make estimations of the TOC (or PMI_{min}) [2]. Development datasets are created for forensically-important species by rearing laboratory colonies at a series of constant temperatures and reporting the time taken for individuals to reach each stage of development [1]. These data can then be used as a direct reference for evidential samples taken from crime scenes, as-

suming that larval samples can be accurately identified to species level. Use of this method has been widely accepted for the past three decades or so, however, researchers are beginning to question its reliability, particularly in the face of criticism from the wider forensic and legal communities [21–23]. It is becoming clear that practitioners can no longer ignore some of the major caveats of degree day models and the creation of development datasets in the laboratory [4, 24, 25]. The rest of this review is dedicated to understanding some of the major factors affecting development of blow flies and the limitations this imposes on the degree day method as an accurate descriptor of PMI_{min} .

1.2 Factors affecting the development of blow flies

1.2.1 Temperature

Life on earth is intrinsically linked to the availability of energy from the sun. It is therefore unsurprising that temperature is fundamental to the development and ultimate survival of every organism on the planet [6]. Although scientists have been historically interested in studying the thermal biologies of organisms (for example: [26], [27]), the advent of the climate change crisis has seen an increase in the effort to understand how the life-history traits of individuals, communities and populations of organisms are affected by changes in temperature and extreme weather events [28, 29]. Ectotherms (including poikilotherms) are particularly important models in these systems due to their ability to survive and reproduce in the face of fluctuating internal body temperature, and it would seem that such plastic life histories are a result of selection in the face of environmental thermal heterogeneity [6].

Unlike endotherms which use homeostasis to maintain an internal thermal constant (e.g., 37°C for humans), an ectotherm’s core temperature relies on environmental heat sources. Specifically, poikilothermic organismic body temperature reflects that of the environment, and they are able to survive in a range of temperatures. These organisms are

still thermally constrained; each organism has a temperature above and below which development will cease and in extreme cases, will be fatal. These thermal limits are the cause of the curvilinear shape of poikilothermic development i.e., where development within the range of the thermal limits follows a linear function, but development at or near the thermal limits is somewhat retarded [1, 30] (Figure 1.1).

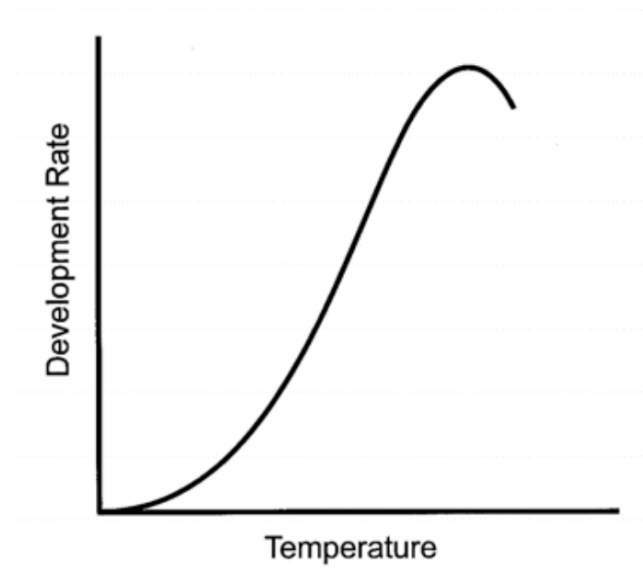


Figure 1.1: Basic curvilinear representation of insect development, taken from Haskell and Higley [1]

The true influence of temperature on insect growth still alludes the scientific community [1]. Berrigan [31] noted that temperature is a puzzle for life historians since it has a counterintuitive effect on growth and size. High degrees of variation among and between species of insects pose a challenge to researchers looking for a unifying theory. Early work focused on the degree day approach, borrowed from agricultural researchers interested in crop development. This form of heat summation focuses on the linear portion of

the development rate curve, falling in between the thermal limits [32, 33]. Although this model is widely used, notably in crop management, Integrated Pest Management (IPM) and forensic entomology, it is far from the most accurate and has been widely criticised [4, 24, 30, 34, 35].

In response to these criticisms, many attempts were made to mathematically describe temperature-based development which included development at or near the thermal limits (a detailed review of these can be found in [30]). The Logan [36] model stands out as one of the most influential during this period - this model combines two separate functions to describe development above and below the optimum temperature. However, Shi et al. [37] note that since the model does not cross the x-axis, it cannot predict the lower development threshold and as such the linear model is required alongside it to get a full picture of development.

One of the most influential ideas has been the biophysical model. Growth and development, like other physiological functions of organisms, are based on biochemical reactions within the body. Certain conditions are required in order for these processes to move forward; for example, enzymes will denature at very high temperatures or will fail to bind with substrates at lower temperatures due to a lack of energy availability. Sharpe and DeMichele [38] were the first to formally propose that poikilothermic development rate was a consequence of such rate-limiting enzymes and subsequently a set of formal models were proposed based on this biophysical system [30, 39]. However, Haskell and Higley [1] warned that although these models have proven very helpful, they focus only on the notion of rate-limiting enzymes and therefore reject many of the other possible factors contributing to temperature-dependent development.

A more recent debate has focused on the metabolic theory of ecology. Thought to be a driver of pattern and process in general ecology, this theory predicts metabolic rates drive survival, development and reproduction across all levels of biological organisation [29].

In terms of temperature-dependent development, there is thought to be a universal temperature dependence for all organisms, being based on an Arrhenius equation [40]. However, arguments around the ecological relevance and constancy of some components of the universal temperature dependence suggest that this too falls short of fully explaining insect development in terms of temperature [37].

Temperature is the main focus of the development literature relating to forensic entomology. A summary of the existing development data for common blow flies in North America is given in Table 1 as an example of the breadth of published studies.

1.2.2 Resource availability and nutritional quality

Food availability and adequate nutrition are vital to the survival of all organisms. For the insects, the choice of food and its availability within the ecosystem impacts life history traits, including growth and development [71]. Indeed, the life cycle of any given species is often closely related to the abundance and availability of its food resource [72]. Exploitation of more widely available foods such as plants, is typically associated with a trade-off with nutrient quality and as such, development time is longer to account for the requirement to spend more time foraging. However, insects exploiting high nutrient, ephemeral resources such as carrion, have extremely fast development times and when that resource is abundant, juveniles may allocate more resources to growth resulting in larger mean body sizes [73, 74].

Nutritional quality and availability of a food resource is of particular interest to forensic researchers. Although a piece of carrion may offer an abundance of food for a larva, if the carrion is old and has undergone prolonged decomposition, the nutrient quality may be less than optimal. This was demonstrated in a study by Richards et al. [75] who found that decomposed liver retarded development times in *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) when compared with cohorts reared on fresh or ground pork. One

Table 1.1: Summary of development data and location of study for several species of forensically-important blow flies in North America

Species	Source data (Location of study)
<i>Chrysomya rufifacies</i> (Macquart)	Levot et al. (New South Wales, Australia) [41], Byrd and Butler (Florida, USA) [42], Sukontason et al. (Chiang Mai, Thailand) [43], O’Flynn (Queensland, Australia) [44], Abdalgalil (Maharashtra, India) [45], Flores et al. (Texas, USA) [46]
<i>Chrysomya megacephala</i> (Fabricius)	Levot et al. (New South Wales, Australia) [41], Gabre et al. (Cairo, Egypt) [47], Thyssen et al. (Sao Paulo, Brazil) [48], Sukontason et al. (Chiang Mai, Thailand) [43]
<i>Cochliomyia macellaria</i> (Fabricius)	Boatright and Tomberlin (Texas, USA) [49], Cunha-e-Silva et al. (Bahia, Brazil) [50], Byrd and Butler (Florida, USA) [51]
<i>Lucilia sericata</i> (Meigen)	Evans (unknown) [52], Davies (Durham, UK) [53], Kamal (Colorado, USA) [54], Ash and Greenberg (Chicago, USA) [55], Nuorteva (unknown) [56], Davies and Ratcliffe (Durham, UK) [57], Greenberg (Chicago, USA) [58], Anderson (British Columbia, Canada) [59], Grassberger and Reiter (Vienna, Austria)[60], Marchenko (St. Petersburg, Russia) [61], Shiravi et al. (Tehran, Iran) [62]
<i>Lucilia cuprina</i> (Wiedemann)	Levot et al. (New South Wales, Australia) [41], O’Flynn (Queensland, Australia) [44], Dallwitz (Canberra, Australia) [27], Day (New South Wales, Australia) [63]
<i>Phormia regina</i> (Meigen)	Kamal (Chicago, USA) [54], Nishida (Japan) [64], Greenberg (Chicago, USA) [58], Greenberg and Tantawi (Chicago, USA) [65], Anderson (British Columbia, Canada) [59], Byrd and Allen (Florida, USA) [66], Marchenko (St. Petersburg, Russia) [61], Nabity et al. (Nebraska, USA) [67], Nunez-Vasqueth (Texas, USA) [68]
<i>Calliphora vicina</i> (Robineau-Desvoidy)	Evans (unknown) [52], Kamal (Colorado, USA) [54], Nuorteva (unknown) [56], Davies and Ratcliffe (Durham, UK) [57], Reiter (Wein, Austria) [69], Greenberg (Chicago, USA) [58], Anderson (British Columbia, Canada)[59], Marchenko (St. Petersburg, Russia) [61], Donovan et al. (London, UK) [70]
<i>Calliphora vomitoria</i> (Linnaeus)	Kamal (Chicago, USA) [54]

of the important points here is about microbial decomposition which affects the quality of resources and can additionally act as a competitor to higher organisms [76]. Likewise, In another study of *C. vicina*, Kaneshrajah and Turner [77] found that development times for this species were faster by up to two days when reared on pork brain, heart, kidney or lung compared with pork liver. Differential development for was also later confirmed for *C. vicina* between pork brain and pork meat (Cuttiford, *unpublished data*). Moreover, Boatrigh and Tomberlin [49] found no significant difference in weight or length for larvae of *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) when reared on equine muscle and pork muscle, however they did find a difference for developmental time, with larvae taking longer to develop on equine muscle. These findings highlight one of the main parameters of interest for developing models, and a number of the published forensic studies do not account for the ecological relevance of the diet used in rearing cages.

1.2.3 Competition and predation

Interactions occur both within and between species, as well as within and between trophic levels. The types of competition and predation are many across the animal kingdom, however they all have an impact on growth and development of the organisms involved. As with all the factors discussed here, competition between organisms involves trade-offs that impact life history traits [78, 79]. In the face of increasing density of a population, a juvenile may trade-off for smaller adult size, decrease or increase in development rate or face reduced fecundity as an adult.

Competitive interactions among carrion-using insects are well described in the literature [80–87]. Competition is broadly split into three groups: interference competition, exploitation competition and apparent competition. Interference competition is a form of direct competition between individuals leading to an impact on foraging, development or other survival. Exploitation competition occurs when a individual or group of individuals

exploits or uses a resource so that it becomes unpalatable or otherwise unavailable to other individuals. Finally, apparent competition is a type of indirect competition where two or more species are prey of the same predator. VanLaerhoven [88] gives a detailed summary of the types of competition and predation in carrion communities.

In an intriguing experiment, Shiao [84] studied the effect of larval density (i.e., interference competition) on development of single- and mixed-colony populations of *Chrysomya megacephala* Fabricius (Diptera: Calliphoridae) and *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae). Intraspecific competition for food within each of these species resulted in faster development and smaller adult body size, particularly in *C. megacephala*. Interspecific competition between these two species alluded to the ability of *C. rufifacies* to physically eject *C. megacephala* from larval masses and as a result, *C. megacephala* was forced to shorten its larval periods and pupate early. A similar effect of competition on body size was found between *C. vicina* and *Lucilia sericata* Meigen (Diptera: Calliphoridae), the latter suffering reduced development times in mixed cultures compared with single cultures[80]. Likewise, Brundage et al. [87] demonstrated that priority effects between *C. rufifacies* and *C. macellaria* impacted the development, survivorship and fecundity of each species. Such impacts could lead to selection towards differential development traits to allow for coexistence.

Exploitation competition occurs both within and between species groups at carrion as well as across kingdoms. Carrion is a resource exploited by scaling trophic levels of organisms, and studies of interactions between the levels of biological organisation have highlighted some important associations. Two such studies, exploring the relationship between the blow fly *Lucilia sericata* and the bacteria *Proteus mirabilis* (Enterobacteriaceae) found that quorum sensing molecules emitted by the bacteria acted as a cue for the blow fly [18, 89]. When presented with bacteria without the ability to emit the volatile compounds, *L. sericata* were no longer attracted to the resource in pairwise attraction assays.

1.3 Plasticity and the role of genetics in development of carrion-using insects

Most of the intrinsic factors relating to blow fly (and indeed all organismal) development are fundamentally the result of genetics. Research investigating the role of differential gene expression and population genetics is vast and beyond the scope of this paper. However, in the past decade there have been a number of important publications discussing developmental aspects of forensically relevant species from a genetic point of view, as well as investigations into variation at the population level and how understanding these mechanisms may aid forensic practitioners. It could be argued that systems based on genetic models might well be easier to control than the classical ecological ones; although it is likely that a combination of ecological, genetic and evolutionary research will best meet the demands of the modern legal system [2]. Without doubt, information regarding the genetic differences at the individual and population level and how they contribute to developmental plasticity of forensically-important species will be invaluable to reducing error in PMI_{min} estimations.

Angilletta [6] suggested that thermal responses are best described as phenotypic plasticity, which is broadly understood as the ability of one genotype to produce a number of possible phenotypes. Plasticity occurs due to some gene x environment interaction either at the time of development (typically irreversible) or sometimes during adult life (typically reversible) [6, 90, 91]. Most of these responses are usually described using a reaction norm, which plots a continuous environmental variable (such as temperature) against a continuous phenotypic variable (such as age and size at maturity). For example, Figure 1.2 shows the phenotypic response of three theoretical subpopulations across two environments, E1 and E2. Plasticity is described as:

$$\text{Plasticity} = G + E + (G \times E)$$

(1.1)

Where G is the genotype, E is the environment and GxE is the genotype by environment interaction term. Referring to our figure, the effect of genotype at the population level is seen where there are differential slopes in the same direction (slopes a and b). An environmental effect can be seen where there are differential means at both the population and subpopulation level (μ_1 vs. μ_2). Finally, a genotype by environment interaction effect is noted where the slopes cross one another; slope c cross slopes a and b. For comparison, a dotted line represents the assumed response of an organism in the ADH/ADD model, a flat line indicating no genetic effect and no slope indicating no environmental effect. In reality, the natural system is much more complex than the ADH model describes. Reaction norms have certainly proven a very useful tool for understanding optimal conditions for certain organisms and what constraints are imposed on the trait of interest by the environment.

Life history traits are known to be affected by geography throughout the animal kingdom. In an intriguing study of developmental plasticity, Tarone and Foran [91] discussed the rearing conditions of a population of *Lucilia sericata*, comparing a laboratory and field cohort. On comparison with other published data, the authors hypothesised that geographically disparate populations likely had differential development times and furthermore that the variable rearing conditions presented in each publication had an impact on the developmental outcomes. They highlighted the need for forensic studies to control for both the genetic and environmental factors affecting blow fly development. Similarly, Anderson [59] investigated the development of four species of forensically-important blow flies found in British Columbia, Canada. She touched on the need for data relevant to British Columbia as existing development data for those species at the time were based on temperatures not typically experienced there. This dataset was recently subject to blind field

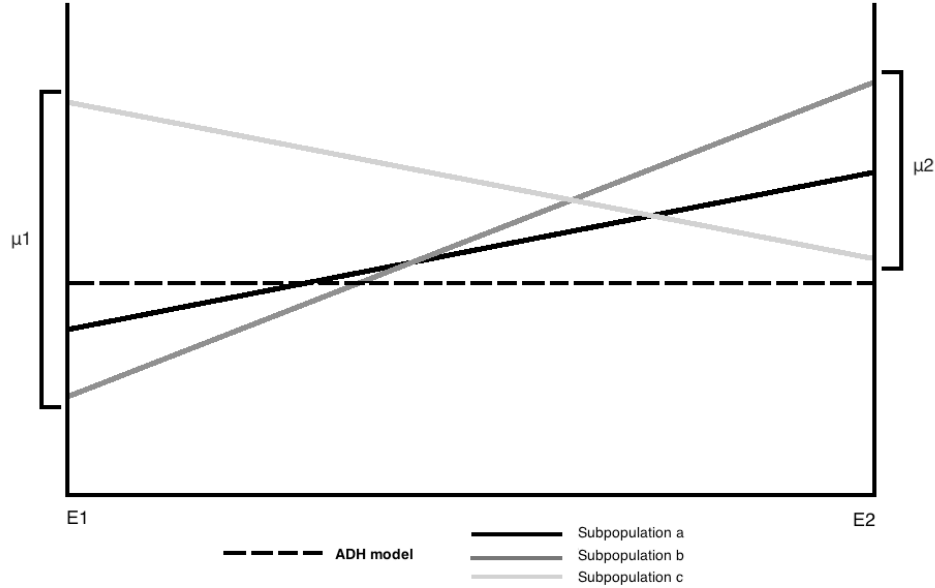


Figure 1.2: Theoretical example of the phenotypic response of three subpopulations (slopes a, b and c) in two environments (E1 and E2) and the response of an ADH model in the same system. Adapted from Tomberlin et al. [2]

validation by VanLaerhoven [92] and was found to be the most accurate predictor of actual PMI_{min} out of five datasets used.

Thermal adaptations and responses to fluctuating environmental temperatures have been a point of interest for thermal biologists for several decades and insects have proven to be a useful model organism for empirical research in the area. Finding a unifying theory to describe developmental outcomes in thermally heterogeneous environments has proven a puzzle for some [31, 93]. Atkinson [94] coined the 'temperature-size rule' which has come to be one of the most prolific theoretical underpinnings of life history theory [6]. The temperature-size rule is a special case of Bergmanns Rule [95] whereby variation in size is due to phenotypic plasticity [96]. Atkinson derived the rule from a review of 109 published studies investigating the effect of temperature on ectothermic organisms spanning animals to protists and plants. He found that approximately 80% of species across

all kingdoms acted within the realm of this rule. Therefore, at lower temperatures, organisms generally develop more slowly and have a larger body size. In warmer temperatures, they develop more quickly and have a smaller body size. Stearns [97] posited that an organism should not mature at a fixed age or size, but instead along an age-size trajectory in a 3-dimensional space. Using this model made it easier to separate the environmental and genotypic variation in developmental responses, something that had previously proven difficult. By using data from several fish species and validating it against data from humans, fruit flies, platyfish and red deer, the results could be used to explain both endo- and ectothermic strategies of thermoregulation.

Sexual dimorphism exists in both genome size and larval length during the third instar in several species of blow flies [91, 98, 99]. Moreover, it has been demonstrated that developmental plasticity exists within genetically distinct populations [100]. Such findings highlight that variability in development happens across population-level scales, an important source of potential error when estimating PMI_{min} . Experimental parameters can be improved to reduce or control for variability in laboratory populations when its fundamental basis is understood.

1.4 Degree day models and insect development

“Pattern implies some sort of repetition and the existence of repetition implies that some prediction is possible” - *Robert MacArthur*

The relationship between insect development and temperature is such that as temperature increases, so does the insect metabolic rate. This relationship is the basis of the heat unit system of measurement, which is known as the degree day, day degree or degree hour. This can be summarised or accumulated to indicate the growth period of an organism under various climatic conditions, which ultimately affect the metabolic rate. Although there are hundreds of examples of studies and publications regarding the relationship of devel-

opment to temperature in ectotherms (for example: [1, 4, 6, 32, 38, 49, 51, 101–112]), the modern degree day model, as it is used in forensic entomology, began to take shape around the 1950s. In two notable publications, Arnold [32, 102] outlined some of the main caveats of the model along with some posited solutions from the literature. He also determined the use of a degree day calculation using maximum and minimum temperatures in sweetcorn cropping. His main contribution was to review some of the basic parameters of the degree day model which acted as a platform to refine the method and enabled researchers outside of cropping systems to apply it to their own fields. Arnold attempted to minimise these issues by devising a degree day calculation method that included a lower thermal limit and daily maximum and minimum temperatures (Figure 1.3C).

Much of the life history of organisms depends on the chemical reactions associated with metabolism [6, 29, 34]. Many of these reactions are enzymatic and require a set of appropriate conditions within which to take place. As all proteins, enzymes are sensitive to outside stressors, particularly heat which is responsible for the conformation step of the reaction. For poikilothermic organisms, this creates a special kind of problem since their body temperature is regulated by environmental conditions. Thus, it becomes important to retain a body temperature that lies between the bounds of critical thermal limits - these being the temperatures below and above which the organism can develop, function and ultimately, live. Provided that the organism is able to remain within these bounds, enzymes will catalyse reactions within their bodies allowing for movement, growth and development. This heat-mediated response provides a unique way of regulating and therefore measuring growth [34].

“All models are wrong, but some are useful” - *George Box*

The core of the degree day model describes a unit of thermal summation and measures the accumulation of heat experienced by an organism over time. Many variations of the

degree day model exist, each aiming to adjust the model slightly to improve estimations, given variable conditions. Most of these models use the same basic parameters; a thermal minimum and/or thermal maximum, a time unit (e.g., day or hour) and a heat unit (e.g., °C or °F), however they vary in complexity in terms of the underlying mathematical proofing. For example, one of the most popular models in recent history is that of Sharpe and DeMichele [38]. Their biophysical model consolidated work by previous authors and skillfully adapted it to calculate a curvilinear development model which also incorporated upper and lower thermal limits. It is considered to be one of the most flexible models available for measuring development [30] and is still used today to describe ectotherm development [113–115]. In another example, Logan et al. [36] developed a popular model, useful for finding the optimal temperature and upper thermal limits of an organisms. A full account of all the available functions and equations to describe the very complex nature of heat-dependent development is beyond the scope of the current paper, however some good reviews can be found by Wagner [30].

Four linear methods exist to calculate degree days, all of which assume that there is a linear relationship between temperature and development time [3]. Many modern uses of the model include mathematical corrections using complex equations (see [116] and [117] for summaries), however forensic entomologists have yet to make serious headway beyond a few experimental examples [19, 25, 118].

Arnold's [32, 102] sine method with upper and lower threshold limits it outlined above and in Figures 1.3C. Following on from these publications, other researchers began to develop alternative methods of degree day calculations which might account for differences between field sites and organism types. Baskerville and Emin [33] extended Arnold's model, adding an upper thermal limit to their own sine curve model. This became known as the horizontal cutoff, the first of the "cutoff" methods for determining degree days. Additional cutoff methods were outlined by Zalom et al. [119]; the intermediate cutoff

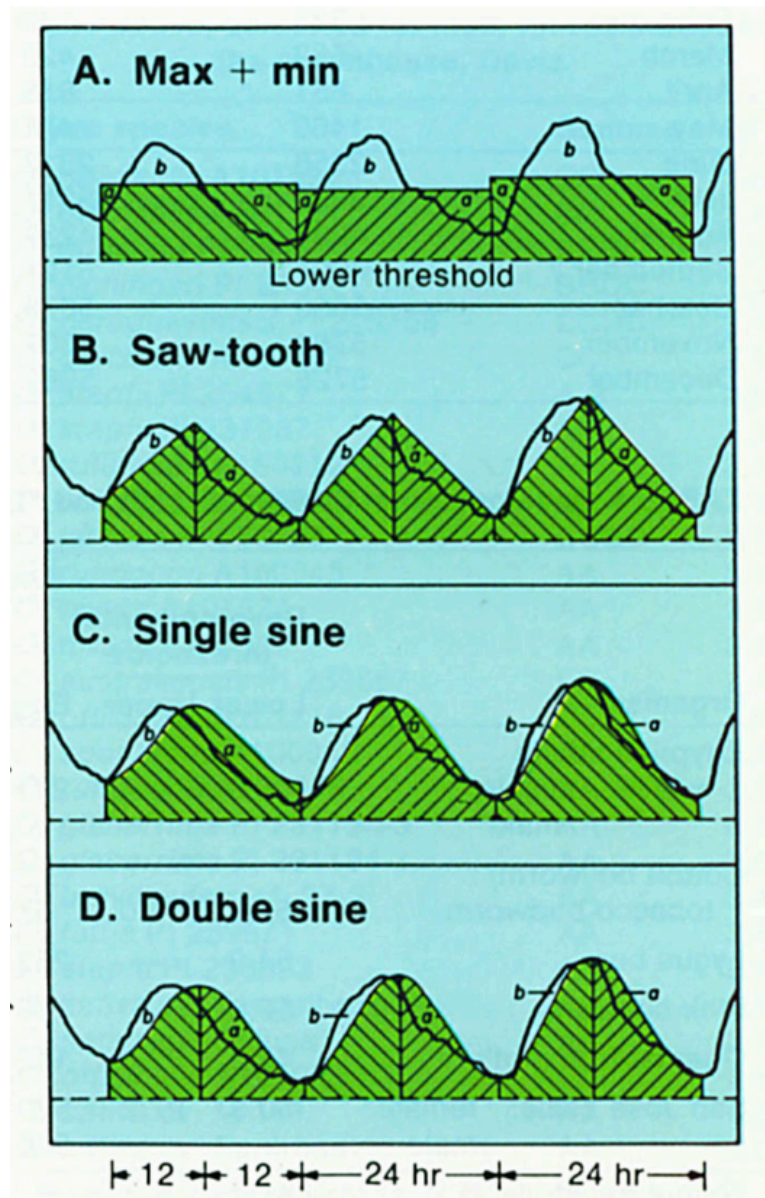


Figure 1.3: The sine method for calculating degree days from diurnal temperatures. The sine wave makes a rough representation of the fluctuation of temperatures across a 24 hour period, underestimating development in the first part of the day (b) but then overestimating in the second part of the day (a), so that a and b cancel each other out, adapted from [3]

method assumes that development only slows, rather than stopping completely at the upper thermal limit and the vertical cutoff method assumes that development stops completely. Methods are all compared in Figure 1.4.

Beyond this, Wilson [3] summarised the four main methods: maximum and minimum, saw tooth method (sometimes referred to as the triangle or trapezoid method), single sine and double sine (Figures 1.3).

1.5 The use and abuse of the degree day concept

“Nature is simple. And it’s the task of the scientist to show that it’s simple; and if we’ve not been able to do that then we’ve failed as scientists. If you find irreducible complexity, you simply haven’t understood.” - *Noam Chomsky on Galileo*

The degree day concept has been used for well over a century and has enjoyed much attention in the scientific literature. Although many papers have described in depth one or two problems with the model, only a handful have given a complete overview of all the caveats. When developing his calculation method, Arnold [32, 102] highlighted some of the issues he found with the model. Firstly, he considered the effect of the method by which the thermal minimum for the organism was calculated. Choosing the wrong base temperature results in an erroneous linear system and the error is cumulative so not only is there discrepancy for one day, but this will add up over the several extra days that could be added or subtracted from the true development rate. Secondly, he noted that poikilothermic development is curvilinear in nature, however the degree day model is a linear model and therefore errors may accumulate in the upper and lower boundaries of temperature limits where development does not follow a linear relationship. Finally, Arnold pointed out the importance of considering factors other than temperature on development, this caveat is one that still causes a great deal of problems for researchers working with field systems as it is often very difficult to account for the amount of variation caused by external factors.

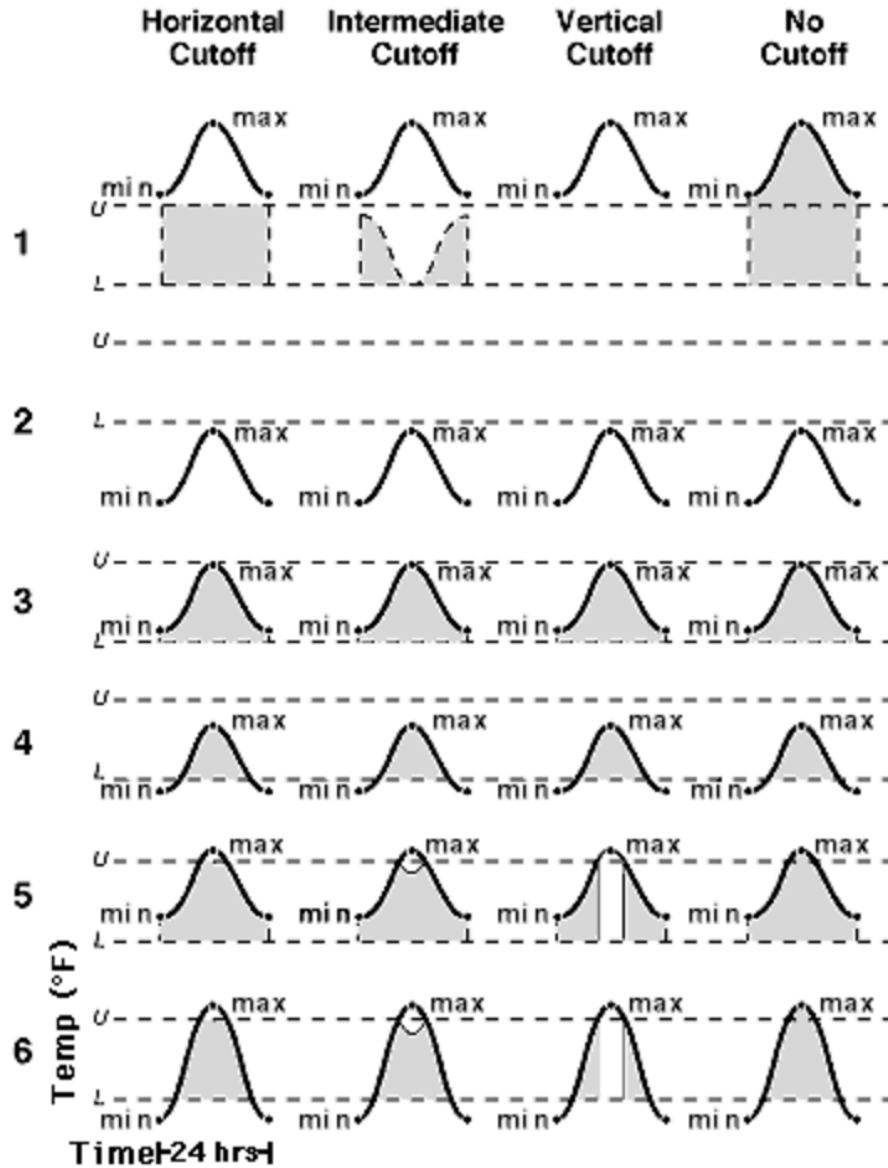


Figure 1.4: Summary of the various cutoff methods. Examples are given of the effect of each of the cutoff methods under six different relationships: 1) Above both thresholds, 2) Below both thresholds, 3) Between both thresholds, 4) Intercepted by the lower threshold, 5) Intercepted by the upper threshold and 6) Intercepted by both thresholds. Adapted from UC Davis IPM website (ipm.ucanr.edu)

The most recent comprehensive review by far is by Bonhomme (2000) who summarised the four main areas of concern. Namely, the influence of non-linearity, the method used to define the thermal thresholds, the quality and accuracy of the reference temperatures used and the influence of external factors on development. These are included in the following discussion, with exception to external factors, which are discussed in the previous sections.

1.5.1 The Kaufmann effect

The Kaufmann effect is a form of the rate summation effect used to specifically describe non-linear, temperature-dependent development [120, 121]. At low temperatures, fluctuations above and below the mean temperature produce a development rate greater than that produced by the true mean due to the rate of development increase above the mean being larger than the development decrease below the mean. The reverse is true for high temperatures; fluctuations produce a development rate lower than that produced by the true mean. In practical terms, this means that data produced from development taking place near to the thermal limits of a given organism will be much less accurate than that derived from development nearer the centre of the thermal range. Subsequently, the accumulation (or summation) of the degree days or degree hours calculated over the course of the model could potentially be quite far removed from the actual developmental requirement. Summation errors such as the Kaufmann effect may be one of the most problematic for the purposes of estimating PMI_{min} , particularly in locations with very variable daily temperatures. Furthermore, they can be linked to many of the other issues associated with degree day models.

In a detailed review of the consequences of the Kaufmann effect on phenological models under variable temperatures, Worner [121] concluded that the closeness of fit between constant temperature data and fluctuating temperature data was so bad as to warrant it

meaningless. She highlighted the difficulties faced by researchers when applying linear models to non-linear development rate functions and how validation and model justification were often erroneously based on laboratory or limited field data. Alarming for forensic practitioners, she theoretically justifies how model selection for development should not be based on how well constant temperature data predicts development within a narrow range of fluctuating temperatures. Moreover, she demonstrates that phenology models (based on the degree day model) that describe more than one life stage are particularly sensitive to small differences in prediction, in other words, when the predictions are made over longer periods of time, the Kaufmann effect will result in predictions that deviate more from the actual development rate.

Some small efforts have been made in decomposition forensics to derive more holistic models for decomposition processes [19, 25, 118] and succession [122–124]. However there have been no formal attempts to collate recent findings of developmental factors in carrion-using insects in order to describe them more accurately. For example, when considering the modeling efforts of researchers in the IPM field, we find a wealth of publications addressing the myriad phenological factors of crop pests and associated organisms (for example: [125–127]). Although the application of the models is different and often on a much larger scale, the underlying mathematics is almost identical. Phenological models in IPM systems are subject to constant updates and additions as researchers discover more about insects and local climate changes. All of these factors also affect research in decomposition systems and heed should be taken to the systematic improvements that are commonplace in closely related fields.

Finally, although Worner make some very serious points regarding the Kaufmann effect, a number of recent papers appear to overlook their significance. In fact, at the time of writing, a manuscript was published regarding the development of *Lucilia illustris* Meigen (Diptera: Calliphoridae) which made a model for forensic purposes based solely on con-

stant laboratory data [128].

1.5.2 Constant vs. fluctuating temperatures

To extrapolate on the causes of rate summation errors in PMI_{min} estimations, the largest source of this error arises from the differential developmental responses of organisms to constant and fluctuating temperatures. Laboratory development studies are, on the most part, carried out at a range of constant temperatures and then applied to field systems which inherently experience fluctuations, some of which may be vastly different to those experienced in an incubator [112]. Moreover, these studies tend not to use temperatures in the range of the critical thermal limits. In fact, differences in development rates have been observed within single incubators, highlighting the high degree of sensitivity in plastic responses within single populations [67]. Likewise, in a study of seventeen different species, Hagstrum and Milliken [112] found that models developed from constant laboratory temperatures were poor predictors in all cases. They highlighted that the degree day model fails to account for temperature fluctuations and demonstrated that development of the red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) at constant temperatures was significantly longer at lower temperatures and shorter at higher temperatures when compared with fluctuating temperatures with similar means. Furthermore, they found that for fluctuations of more than 7°C, the percent deviation from development at constant temperatures was much greater.

Ruel and Ayres [129] discussed the effect of temperature variance (i.e., fluctuating temperature) on poikilothermic animals. They hypothesised that the metabolic rates of organisms would vary not only in different habitat types (e.g., aquatic vs terrestrial) but also by the amount of fluctuation in operative temperatures. Changes in operative temperatures between 3-8°C could see fluctuations in metabolism of 3-50% leading to phenotypic responses in later generations, further muddying the waters of a consistent and

measurable response to thermal heterogeneity. In terms of the impact on developmental models for forensic science, this hypothesis highlights an important factor when using constant temperature data to predict field systems.

Again, Worner [121] lays a solid foundation for understanding the perils of comparing constant and laboratory data for the purposes of deriving predictive models. She highlights the confusion over the real biological effect of fluctuating temperatures on insect development within the literature. Of course, there could well be individual differences at a number of taxonomic levels, however it is clear that those studies based on wider temperature ranges concur that development is generally faster under fluctuating temperature regimes. Another important reason for this confusion relates to the difficulties of comparing linear and non-linear models and, as discussed above, the rate summation effect.

Interestingly, the current methods suggested for use in forensic casework still seek to refine the linear modeling approach by expanding the experimental parameters in constant laboratory data [4]. Greater precision in PMI predictions was found when sample sizes and temporal sampling resolution were increased and, along with a discussion about the various measures of central tendency used in linear modeling, five parameters were suggested. These were 1) using at least five different temperature regimes for development, 2) the use of median as the measure of central tendency, 3) using a range of larval sizes, not just the largest as these represent only the maxima of the population, 4) a sample size of at least thirty specimens per experimental unit and 5) the use of sampling frequencies that are shorter than the developmental events being measured. Guidelines such as these are imperative for forensic practitioners and this paper is certainly the best in the current forensic literature. However there is still a dire need to focus on the use of fluctuating temperature regimes, and perhaps even development of models from field data and furthermore, they should be included in a more comprehensive set of guidelines for general use.

1.5.3 Curvilinear vs. linear models

Although curvilinear models do exist for many species (and indeed have been the mainstay of many phenological models in pest management), a linear model is often used for simplicity. However, it is well established that mapping curvilinear data onto a linear model can cause major systematic errors in estimates [1, 4, 30, 32, 34, 67, 102, 121, 129, 130]. Development rate, as it pertains to temperature, is only linear for the mid-range of the operative temperature of a poikilotherm, with rates declining or increasing near the thermal limits. But despite the existence of several simple mathematical methods for estimating degree days in a non-linear fashion (see [34]), none have been applied to blow fly development for the purposes of estimating PMI_{min} . Even the slightest of improvement in accuracy can make a huge difference in legal proceedings.

Regression analysis (both linear and non-linear) has proven to be the most useful for understanding insect growth, this being a way to estimate the relationship between the variables temperature (independent) and development time (dependent). Data used in regression models must follow a set of general assumptions: independent variables must have zero or negligible variation and should be equally spaced to prevent a skewed relationship as a result of the values at each end of the temperature range [130]. In many cases, problems arise for development modeling when one or more of these assumptions is not met. For example, too few or unequally spaced temperature ranges are frequently used in experiments [4, 130] and by staying safely within the confines of the thermal limits, we see a summation effect on the distal values of the regression curve. Moreover, if a development model is created at a range of temperatures suitable for Chicago, for example, these are unlikely to give an accurate reflection of development rate of the same species Germany or in fact anywhere else. This is in part also due to the different thermal limits used in the regressions and also due to the response to fluctuations in diurnal temperatures

experienced by different populations because such limits are a plastic traits in insects [6].

1.5.4 Method of determining the lower thermal limit (CT_{min})

Several methods have been described for calculating the CT_{min} in a degree day model. The most common approach is the x-intercept method where a development curve is drawn and extrapolated downwards until it meets the x-axis of the plot (Arnold, 1959). This method has some obvious drawbacks including that it is based on temperature means used and therefore could already be erroneous [4]. However, Nabity et al. [67] argue that any value other than that calculated using this method in a degree day calculation will invalidate the core assumption of linearity.

Critical upper and lower thermal limits can vary considerably both within and between species and, if not properly controlled for, can lead to erroneous outcomes for a model. Typically, single CT_{min} is utilised when developing a model and although there are some exceptions, much of the forensic literature fails to either calculate the CT_{min} within the model data or to make their own replicated field observations. Furthermore, it has been noted that the CT_{min} within a degree day model may have no real biological relation to the actual CT_{min} of an individual organism [30, 121]. Likewise, [34] highlighted that estimate accuracy is only weakened slightly when the estimated CT_{min} is lower than the actual CT_{min} , but if the estimated CT_{min} is higher than the actual, accuracy quickly declines.

1.5.5 Representational quality of reference temperatures

Common practice when creating degree day models include the use of historical weather data, typically gathered from online sources such as the National Climatic Data Center (NCDC) (www.ncdc.noaa.gov) and Weather Underground (www.wunderground.com) in the USA [1, 7, 92, 131]. Moreover, sources such as these have in recent years begun to report various degree day values, for example the heating and cooling degree days available from the NCDC. Typically, temperatures are reported as hourly averages, however the

averages may be calculated using variable temporal sampling resolutions across a day or may only be based on one or two readings per hour. These inconsistencies are likely due to the variability in weather station function as well as corrupted data files and other missing information being uploaded to the central database. Another caveat of using these historical data might be the location of the weather station from the actual site of interest; some study sites may be furnished with their own weather stations, however a "local" weather station may be more than 10km away (see [132]). Depending on the scale of the study in question, the temperature readings from a weather station even one or two kilometres away may be inaccurate enough to cause a meaningful error in developmental predictions [133]. One method of reducing this kind of error involves the use of portable data loggers that can be fitted to equipment, or in the case of carrion studies, put on or near the remains. Several loggers can be used simultaneously to measure subtle differences between the ambient temperature and various locations on the carrion [131–133].

Unfortunately, in forensic casework, it is not possible to retroactively place data loggers at remains, so historical data must be depended on. However, in an important pair of studies, Archer [133] and Johnson et al. [132] investigated the effect of correcting local weather station data on both hypothetical and real cases of remains discovery in Australia. By regressing readings from a data logger placed *in situ* at the site of remains for a period following discovery and those found from local weather stations, these authors were able to make PMI_{min} estimations and evaluate their efficacy. [133] recorded temperature using data loggers at four periods in six locations across Victoria, Australia. Each site produced one hypothetical decomposition period of 10 days and three subsequent 'correlation periods'. All four periods for each site were regressed against temperature readings from a single local weather station to produce a total of 24 linear regression equations. Each of the correction period equations were used to correct weather station data corresponding to the hypothetical decomposition period and were compared to the data collected using the

data logger during that same period. In 22 of the 24 cases, corrected temperature data was closer to the actual temperature collected from the data logger than that collected from the weather station. Building on this technique, Johnson et al. [132] tested the length of the correlation period (2, 5 or 10 days), distance between death scene and weather station (between 2-14.7km) and the temporal sampling resolution of ambient temperature measurements (30 mins, 3 hrs and bi-daily readings). The experimental findings were applied make corrected PMI_{min} estimations in 27 real cases to evaluate outcomes. They concluded that the accuracy of the predictions they made were not significantly affected by any of the experimental parameters, however they warned that differences of $>5^{\circ}\text{C}$ between correlation data and weather station data did produce less accurate temperature readings in some cases. This pair of studies highlighted an extremely important aspect of degree day modeling in forensic entomology as well as a robust validation and evaluation of the methodology for future casework scenarios.

1.6 Evaluation, not validation

“Here we are back again at the question of the meanings of words.” - *Arthur Tansley*

Naomi Oreskes has written extensively on the value of models in biology and the ways in which they are often erroneously portrayed as perfect reflections of the systems they describe [134–137]. Much of her work warns against the tendency to use the term validation, which, theoretically speaking, is an impossibility when creating a model; the word valid implies a form of certainty that a model cannot be expected to produce. In ecological and biological sciences, validation is understood as a means of testing the predictive power of the model (e.g., how well it reflects the system it is illustrating). However, validation cannot fully prepare a model to be used in many real-world scenarios for the model itself is based on a set of assumptions and ultimately a limited set of knowledge about the system. In other words, the inherent complexity of natural systems, coupled with an ever limited

understanding by humans ultimately leads to models which are always, on some level, inaccurate. Conversely, evaluation of a model allows a researcher to uncover hidden issues that the model and initial data could never have predicted; external factors not accounted for or perhaps issues with converting laboratory data to field data. With evaluation, several real-world or field data can be used to test the predictive accuracy of the model and subsequent novel information can be incorporated into the model, making it that much better. In this sense, there are many dangers to relying on models which have been validated, but have not been properly evaluated. This is even more pertinent when models for field data have been created using experimental laboratory data. In forensic entomology, the vast majority of published development data is based on laboratory cohorts of insects reared at constant temperature, humidity and L:D cycles [4]. On the most part, this has no bearing on ambient climate conditions and has been shown to have a serious impact on the ability of that development data to make accurate predictions [25, 30]. This is an important consideration of the pitfalls of improper model evaluation.

Although this could be seen as a matter of semantics, language is extremely important - particularly so in young field still finding it's feet [138–140]. In fact the title of this thesis pays homage to the seminal piece by Tansley [141], in which he discusses at great length the importance of precisely defining words used to describe ecological systems, as well as being humble about the limitations of human modeling efforts of natural systems. Separating the words validation and evaluation will give researchers a clearer roadmap for future work and denotes two distinct steps in model production for evidential analysis. Creating solid conventions grounded in basic research and theory for forensic entomology will go far to meet the demands of the criminal justice system [2, 22, 23].

1.7 *Cochliomyia macellaria*

The secondary screwworm, *Cochliomyia macellaria*, is a purely saprophagous blow fly and one of the most commonly distributed in North and South America [85]. It has relevance for both human and livestock health and is a primary agent of facultative myiasis. It also contributes to the proliferation of disease-causing agents due to mechanical pathogen transmission [49, 85]. Moreover, it is a common species of forensic importance in the northern USA as it is attracted to decaying flesh, feeding, mating and eventually ovipositing on carrion. Larvae of this species have been used in forensic casework and laboratory development data exist from Texas, Florida and Brazil (See Table ??). Morphological characteristics for identification are established in the literature [142], making them an excellent model organism for the current study.

1.8 Objectives and hypotheses

The objectives of the research are as follows:

1. Determine the accuracy of estimating time of placement (TOP) of human remains in the field using *C. macellaria* development data to make estimates of age of larval samples from human remains H_0 : There will be no difference between predicted TOP and actual TOP across all development datasets. H_a : There will be significant differences between predicted TOP and actual TOP across all development datasets.

Relevance: Validation of methodologies used for evaluating evidence in the forensic sciences is lacking, in large part due to the top-down nature of case work; evidence is presented to practitioners who then must devise the best scientific theory to apply. This is unlike other areas of science where effort is focused on developing techniques and theory to aid in the answering of existing hypotheses, a bottom-up approach. Due to the relative infancy of forensic entomology more specifically, much work is required to improve the robustness of time interval estimations such as TOP and postmortem interval. This study

aims to be the first extensive validation of laboratory rearing data of blow flies in North America and beyond and will answer directly the concerns of the National [23]. To the author's knowledge there have been no previous studies that have attempted to address this issue through the use of known real-time samples taken from human remains. Additionally, we will use the real-time data to make TOP estimates of blow fly samples from swine remains in Maryland, USA and Cologne, Germany. This will have two main outcomes - a further validation of the accuracy and precision of the existing data and a brief investigation into the regional fluctuations of blow fly development on carrion.

c. Assess intra-specific variation between seasons. H_0 : Season will not influence the accuracy of TOP predictions made using published development data. H_a : Season will significantly influence the accuracy of TOP predictions made using published development data.

Relevance: Since response to temperature and climatic changes is known to be a plastic response in insects, it can be reasonably hypothesised that changes in season could have differential impacts on developmental processes. If season does influence development rate, then this finding will act as yet another facet in future models, improving their predictive power for casework.

2. FIELD STUDY: BLIND FIELD EVALUATION OF *COCHLIOMYIA MACELLARIA* DEVELOPMENT DATASETS

2.1 Introduction

Historically, forensic entomologists have used laboratory developmental datasets to make estimations of time of colonisation (TOC) of human remains [7, 35]. Most commonly used in cases of homicide or other suspicious deaths, estimations of the TOC (commonly referred to as the minimum Postmortem Interval (PMI_{min})) [143] are derived by comparing average phenotypic traits, such as time to reach specific developmental thresholds, between field samples and laboratory data. Although there are a handful of exploratory studies (see [91, 143, 144], traditional PMI_{min} estimations in forensic entomology are based on the degree day or accumulated degree day (ADD) or hour (ADH) model of insect development. This model is linear in nature and describes temperature-dependent development of a poikilothermic organism within the boundaries of its upper and lower thermal limits, beyond which development is assumed to cease [102].

A number of serious problems exist for the ADH/ADD model and this is reflected in the erroneous ways that developmental data are created in the laboratory environment and the subsequent issues they cause for statistical inference. In a thorough critique of forensic entomology methods, Michaud et al. [25] argue that the practice of using single growth chambers as experimental units can lead to both biological and statistical problems with development experiments. For instance, replicating the same temperature treatment within a single growth chamber leads to simple pseudoreplication. Furthermore, unknown chamber effects (vibrations, heterogeneous temperatures within the chamber) can lead to unquantifiable variation within the subsequent development model. Finally, variation such as this could potentially lead to a Type I error where variation is detected, but it is not

due to the effect of temperature, the treatment under investigation. A number of recent studies [46, 67, 68, 145] recognising the issue of within-chamber effects opted to use a Latin square or randomised block design, assigning chambers and samples randomly for each trial replication. Interestingly though, none of these studies utilised the mixed model analysis that was recommended by Michaud et al. [25] to extract the block effect for each trial.

In addition to some of the more practical problems when developing ADH models from laboratory data, there are a number of theoretical considerations. Laboratory development studies are, on the most part, carried out at a range of constant temperatures and then applied to field systems which inherently experience diel fluctuations, some of which may be vastly different to those experienced in an incubator [25, 67, 112]. This effect of constant vs. fluctuating temperatures has been well demonstrated in applied entomological literature [27, 109, 112, 121, 146], which reports differential development outcomes during fluctuating temperatures (i.e., either faster or slower development) at the species level, further muddying the waters. Moreover, many studies (for example: [46, 49]) tend not to use temperatures that include those near the upper and lower thermal limits of the study organism, which reduces the predictive power of the model [4].

Discrepancies between the experimental and actualised developmental temperatures can amplify the Kaufmann effect, producing models that have development rates that are much slower or much faster than natural populations. The Kaufmann or rate summation effect is a form of the rate summation effect used to specifically describe non-linear, temperature-dependent development [120, 121]. At low temperatures, fluctuations above and below the mean temperature produce a development rate greater than that produced by the true mean. This is due to the rate of development increase above the mean being larger than the development decrease below the mean. The reverse is true for high temperatures; fluctuations around the upper threshold result in a development rate lower than that

produced by the true mean. Subsequently, the accumulation (or summation) of the degree days or degree hours calculated over the course of the model could potentially be quite far removed from the actual developmental requirement.

The application of a linear model (ADH) to a curvilinear pattern (most insect development) also poses problems for TOC estimations. Although curvilinear models do exist for many species (and indeed have been the mainstay of many phenological models in pest management), a linear model is often used for simplicity. However, it is well established that mapping curvilinear data onto a linear model can cause major systematic errors in estimates [1, 4, 30, 32, 34, 67, 102, 121, 129, 130]. Development rate, as it pertains to temperature, is only linear for the mid-range of the operative temperature of a poikilotherm, with rates declining or increasing near the thermal limits.

Other problems with the ADH model include the quality of the reference data (i.e. historical weather data) used to make the estimations and the method used to derive the upper and lower thermal limits of the organism. Historical weather data may be from stations that are far away from the actual site in question; a "local" weather station may be more than 10km away (see [132]). Depending on the scale of the study in question, the temperature readings from a weather station even one or two kilometres away may be inaccurate enough to cause a meaningful error in developmental predictions [133]. Likewise the upper and lower thermal limits of an organism, may have been derived from the laboratory model itself rather than from observations or measurements made in natural populations. Nabity et al. [67] argue that any value other than that calculated using this method in a degree day calculation will invalidate the core assumption of linearity.

Recently there have been calls for better validation and evaluation of the methods and models used in the forensic sciences [22, 23]. Therefore a more rigorous approach to creating ecological and biological models for evidential analysis is crucial to the future of the field [22, 144]. Recognising the distinction between validation and evaluation is

critical- validation in the classical sense relates to testing the ability of the model to make accurate predictions of the data used to create it. Evaluation, on the other hand, allows the model to be tested within a larger - perhaps natural - system so that the experimenter is able to shed light on factors affecting the model that may not have been considered or even known within the laboratory setting [135, 136]. Given the complex nature of biological systems, evaluation becomes all the more important.

The secondary screw worm, *Cochliomyia macellaria* (Diptera: Calliphoridae) is a blow fly of established forensic and medical importance. This species regularly colonises wounds and necrotic flesh of both living and deceased animals, causing losses to the livestock industry as well as medical complications. *Cochliomyia macellaria* immatures are regularly collected from human remains in the Southern United States [51, 147] and has been regularly sampled and observed on remains at the Forensic Anthropology Research Facility in San Marcos, Texas, where this study took place. Two development datasets exist for this species using populations from the USA. Byrd and Butler [51] studied a population in Florida at four fluctuating and one constant temperatures, on a diet of lean pork meat. Boatright and Tomberlin [49] used a colony from Texas, which they reared on a diet of either porcine or equine meat at three constant temperatures. The experimental design used in these two studies are similar, although not identical. Furthermore, geographically distinct populations are very likely to have differential phenotypic outcomes and so these studies were analysed both separately and together in order to unpack these. To our knowledge, these datasets have never been subject to field validation.

The current study aims to evaluate the ability of existing datasets pertaining to *C. macellaria* to accurately predict actual TOP of human remains over a three year period in San Marcos, Texas. It is assumed that, when working with blow flies, that TOP is approximately the same as TOC due to the early arrival of these organisms following death, although colonisation may occur antemortem due to myiasis [2, 148]. In casework, estimations can

only be of TOC, as the real TOP of remains is unknown to the investigators and thus as a biological parameter of the estimate. For the current study, however, since the actual TOP was later revealed for the remains, this could be used as our comparison. Three phenotypes were tested from within the datasets - stage (time to third instar), length and weight - to investigate any differences between them. Given the many known caveats regarding ADH models, I hypothesised that the datasets would have limited use in accurately predicting actual TOP of human remains. Additionally, I hypothesised that there would be an effect of season on the ability of the datasets to make TOP predictions.

2.2 Methods

2.2.1 Development data

A thorough search of the literature was carried out and two datasets were chosen for evaluation. These are summarised in Table 2.1, which includes an analysis of the datasets against the five key parameters set forth by Richards and Villet [4] that should be included when designing development data intended to be used for forensic casework. Datasets were chosen as they included full details of the experimental method as well as timings for each developmental stage. Although other publications describe some development data for this species (for example, [50, 58]), none of these included information specifically regarding the phenotypes of interest for this study and so were excluded.

Table 2.1: Summary of datasets for *C. macellaria* used for the current study. * indicates the parameters suggested by Richards and Villet [4] ^a indicates fluctuating temperature mean with amplitudes of 5.5°C and 12:12 L:D. ^b indicates a constant temperature under continuous light. ^c indicates constant temperature under 14:10 L:D

Reference	Location	Diet	Diet amount	> 5 temperatures used? (°C)*
Byrd and Butler [51]	Florida	Pork	2g per larva	No (15.6 ^a , 21.1 ^a , 25 ^b , 26.7 ^a , 32.2 ^a)
Boatright and Tomberlin [49]	Texas	Pork and Equine	200g	No (20.8 ^c , 24.3 ^c , 28.2 ^c)
R.H. (%)	Sampling interval (hrs)*	Largest larvae only?*	Replicate sample size*	Parameters reported*
75	No (12)	Yes	Yes (approx. 300)	Mean only
75	No (12)	No	Yes (approx. 400)	Minimum \pm S.E.

2.2.2 Study site and experimental protocol

Field data were collected from human remains placed at the University of Texas Forensic Anthropology Research Facility (FARF), San Marcos, Texas. This site is a by-donation human decomposition research facility used primarily for physical anthropology and taphonomic research. Donated bodies are typically transported to the facility and stored in a cooler until they can be placed on site. Once placed, remains are secured underneath a welded, mesh cage to exclude small mammals, vultures and other vertebrate scavengers in order to maximize the undisturbed study of decomposition and entomological activity. Researchers and students working at the site check the remains daily, but disturbance is kept to a minimum and bodies typically remain untouched for the duration of decomposition.

An application (ADH monitor) was developed to calculate Accumulated Degree Hours (ADH) for the study site in real time. This application exports data from an online meteorological and weather information system, Forecast.io (www.forecast.io), on an hourly basis. This service accumulates weather information from a cluster of local, national (including National Climatic Data Center) and amateur weather stations and condenses them into a single, average reading for the chosen location, giving the most accurate prediction possible for a given area. The application then calculates ADH using a pre-determined baseline temperature (in this case 10°C) and can notify the user when the pre-determined milestones will be reached (in this case 1400, 2000, 2400 and 3000ADH). As the temperature data is updated on an hourly basis through Forecast, the user receives a notice (via email) before the given milestone in order to have time to get to the site. This application is self-validating at each data collection point.

All larval samples were collected using a strict experimental protocol. In brief, collaborators would use the ADH monitor as a notification system to enable them to collect at the given ADH milestones following placement of remains in the field. At these times,

a thorough search of the remains was carried out, checking on and around the body for larval masses. All larvae were transported directly to a lab, hot water killed and preserved in 80% ethanol (as per standard practice [149]). A vial for each sampling point (four total for each set of remains) was labeled and photographs were also taken at each sampling point using a DSLR camera (Canon EOS 70D, Canon, USA).

2.2.3 Sample processing

Larval samples retained in alcohol were all processed at the Forensic Laboratory for Investigative Entomological Sciences (FLIES) facility, Texas A&M University. Each larva was removed from ethanol and first identified under a 4x light microscope (EMZ-STR, Meiji Techno, Japan) using the CDC morphological key for forensically important Dipteran larvae. Specimens of first or second instar were recorded by their development stage only, except in the case of *Chrysomya rufifacies*; this species being extremely distinctive from other congeners. Length measurements were made by photographing larvae under 0.7x magnification using a USB camera (Infinity Capture, Lumenera Corporation, Canada). Software (Infinity Analyze, Lumenera Corporation, Canada) was employed to make repeatable scaled measurements from the photographs. Briefly, a scale of 10mm at 0.7x magnification was preset into the software as a baseline. The internal measuring tool in the software uses this scale to make measurements on images taken using the 0.7x preset. A line measuring tool was used to draw a line along the centre of the body of each larva from spiracular plate to the tip of the mouth hooks. Once length was measured, larvae were transferred to a set of balance scales (Ohaus Corporation, USA) set to 4 decimal places and weighed. The balances were tared between each weighing event. Once both weight and length measurements were collected, the larvae were returned to ethanol for long-term storage.

2.2.4 Time of placement estimations and prediction accuracy analysis

Time of placement estimations were calculated using the averaging model of ADD development [102], with a minimum thermal threshold of 10°C [49]. Maggot mass temperature was not included in any of the analyses. Each development treatment (i.e. diet source and temperature) in Boatright and Tomberlin [49] and Byrd and Butler [51] was used to make an estimate for TOP for each sample that included larvae of *C. macellaria*. Furthermore, TOP estimations were calculated using larval developmental stage (time to 3rd instar), length and weight. Where the developmental data provided a minimum and maximum time to stage or length/weight, these were used to calculate a range, otherwise the mean was used to create a single point estimate. Each of these calculations were compared with the actual TOP. Constructing the estimations in table form allowed for a visually meaningful dataset where it was immediately obvious whether a PMI estimation included the actual time of placement. Such constructions have been previously noted as useful for juries in courtroom scenarios and casework [150].

The three different estimation phenotypes - stage (time to third instar), length and weight - were each tested for predictive value. Moreover, each of these phenotypes was tested within different groupings of the data to provide a deeper analysis.

2.2.5 Estimations by larval development stage

The ADH timing of all samples containing third instar *C. macellaria* (i.e. the actual ADH from actual TOP) were compared with the estimated TOP for each publication/treatment combination. Ranges were calculated when maximum and minimum data were available, otherwise a point mean estimation was calculated and the minimum developmental mean was used for prediction calculations. Subsequently, a matrix of TOP estimates based on the reported ADH values was constructed for each sample/publication/treatment combination. Deviations from the actual TOP were then calcu-

lated along with percentage coverage of each estimated TOP (i.e. the amount of the actual TOP that was covered by the estimated TOP).

A two-way ANOVA ($p < 0.05$) was carried out with Tukey's HSD *post-hoc* test, with Percent coverage as the dependent variable and Body ID and Treatment as factors, separated by Milestone.

2.2.6 Estimations by larval length and weight

Lengths and weights of all *C. macellaria* larvae in each sample were averaged with confidence intervals to test the accuracy of published length data in both Boatright and Tomberlin[49] and Byrd and Butler [51] and weight data published in Boatright and Tomberlin [49]. The minimum and maximum values of the confidence intervals were read from the graphs and the hourly values were converted to ADH. Subsequently, a matrix of TOP estimates based on the reported ADH values was constructed for each sample/publication/treatment combination. Minimum and maximum deviations from the actual TOP were then calculated along with percentage coverage of each estimated TOP (i.e. the amount of the actual TOP that was covered by the estimated TOP).

A two-way ANOVA ($p < 0.05$) was carried out with Tukey's HSD *post-hoc* test, with maximum percent coverage as the dependent variable and Body ID and Treatment as factors, separated by Milestone. Maximum values were chosen as these represented the oldest possible age of the larvae, a common practice in casework estimations.

2.2.7 Effect of season

I was interested in the possible influence of season on prediction accuracy of the development data. As such, samples were divided into four seasons, autumn (September - November), winter (December - February), Spring (March-May) and Summer (June-August). These groupings were largely arbitrary and used to evenly divide the year, however they are considered to reflect the changes in temperature during an average year in

Texas.

A two-way full factorial ANOVA was carried out to assess the effect of season on the stage, length and weight phenotype data. The factors used were season and treatment and the interaction term season x treatment, with percentage coverage as the independent variable and results being divided by milestone.

2.3 Results

2.3.1 Collection summary and temperature data

Larval samples were collected from a total of 29 sets of remains between September 2013 and June 2016 (Table 2.2), culminating in a total of 116 samples (four per set of remains). Of these, a total of 80 samples across 26 sets of remains contained 3rd instar larvae of *C. macellaria*.

Table 2.2: Summary of all human by-will donations utilised for sampling over the period September 2013 - June 2016 at FARF

Donation	Sex	Age	Height	Weight	Place of Death	Storage	Time of Placement
D50-2013	M	71	182.88	160	Home of Deceased	Cooler on 9/20	9/21/13
D51-2013	M	75	166.37	170	Home of Deceased	right to surface	9/22/13
D53-2013	M	65	177.8	148	Hospital Inpatient	wrapped in a carpet @ 5pm on 10/9	10/11/13
D54-2013	F	94	152.4	113	Nursing Home	wrapped in carpet @ 11am on 10/10	10/11/13
D23-2015	M	69	170.18	170	Home of Deceased	Freezer 5:45pm 5/14/2015	6/8/15
D24-2015	F	67	149.9	150	Nursing Home	cooler 6:05pm 5/15/2015	6/8/15
D25-2015	F	68	165	96	Home of Deceased	Freezer 3:30pm 5/16/2015	6/8/15
D27-2015	M	70	177.8	225	Home of Deceased	Cooler 5:45pm 5/31/2015	6/5/15
D28-2015	F	76	137.16	200	Hospital Outpatient	Cooler 5:30 6/2/2015	6/4/15
D30-2015	M	86	182.88	167	Nursing Home	right to surface	6/8/15
D35-2015	F	69	170.18	149	Hospital Inpatient	Cooler 2:43pm 7/1/2015	7/2/15
D39-2015	M	85	162.56	120	Hospital Outpatient	right to surface	7/15/15
D40-2015	M	83	167.6	138	Hospital Inpatient	Cooler 5:10pm	8/5/15
D43-2015	F	97	160.02	150	Nursing Home	Cooler 4:45pm 8/11/2015	8/12/15
D45-2015	F	79	170.18	150	Home of Deceased	right to surface	8/28/15
D51-2015	M	59	172.72	101	Nursing Home	Freezer 12:50pm 9/12/2015	9/18/15
D53-2015	F	61	170.2	190	Hospital Outpatient	Cooler 9pm 9/25/2015	10/2/15
D54-2015	F	78	165.1	296	Hospital Outpatient	Cooler 3:45pm 9/27/2015	10/2/15
D61-2015	F	84	165.1	80	Nursing Home	Cooler 6:58pm 11/2/2015	11/6/15
D64-2015	M	54	175	198	Hospital Inpatient	Cooler 9:20pm 12/4/2015 then Freezer 12/4/2015	1/26/16
D65-2015	M	59	179	[missing]	Other/ Parking Lot	Freezer 2:30pm 12/8/2015	1/26/16
D66-2015	F	56	165	141	Nursing Home	Freezer 6:20pm 12/6/2015	1/26/16
D06-2016	F	66	163	126	Hospital Outpatient	right to surface	2/19/16
D08-2016	F	52	161.5	118	Home of Deceased	right to surface	2/29/16
D23-2016	F	74	171.5	301	Home of Deceased	Cooler 12:41pm 6/2/2016	6/16/16
D26-2016	F	86	157.5	89	Nursing Home	Cooler 7pm 6/10/2016	6/21/16
D28-2016	M	83	172	142	[missing]	right to surface	6/21/16
D30-2016	M	50	166	170	Other Home	Cooler 1:10pm 6/21/2016	6/20/16

Figure 2.1 shows the ranges of sampling times for each milestone. Because sampling at the exact milestone was often not possible (for example, the hourly sums didn't fall on the exact milestone or the milestone fell in the middle of the night), the actual sampling times fell within the following ranges: 728.18 - 1551.7 ADH (median 1443.35) for the 1400 ADH milestone, 1870.68 - 2066.88 ADH (median 1973) for the 2000 ADH milestone, 2158.2 - 2570.49 ADH (median 2368.01) for the 2400 ADH milestone and 2890.26 - 3113.02 ADH (median 3021.06) for the 3000 ADH milestone.

Temperature was successfully recorded and real-time ADH calculated by the ADH monitor app for each of the 29 sets of remains. Monthly temperature averages for all three years are shown in Figure 2.2. In addition, the app was used to collect data required to make predictions (e.g., data before the actual TOP) for 2015 and 2016. The same data for 2013 was collected from Weather Underground (www.wunderground.com) as historical data for 2013 was not loaded into the app at the time of analysis.

2.3.2 Time of placement prediction accuracy

Predictions for TOP were calculated for each phenotype and treatment combination in Boatright and Tomberlin [49] and Byrd and Butler [51]. Prediction accuracy was measured by confirming that the actual TOP fell within the range of the predicted TOP. Mean stage accurately predicted twenty nine out of 80 samples, mean weight accurately predicted three samples and mean length predicted only two. The temporal spread of the accurately predicted samples was not meaningful, with stage making accurate predictions in every month represented by the data and no particular month or season being an obvious indicator for either weight or length.

For the purposes of deeper analysis, four samples were removed from the data for having either missing collection information or for being more than 250ADH outside of the sample milestone. Initial prediction tests showed that those samples falling more than

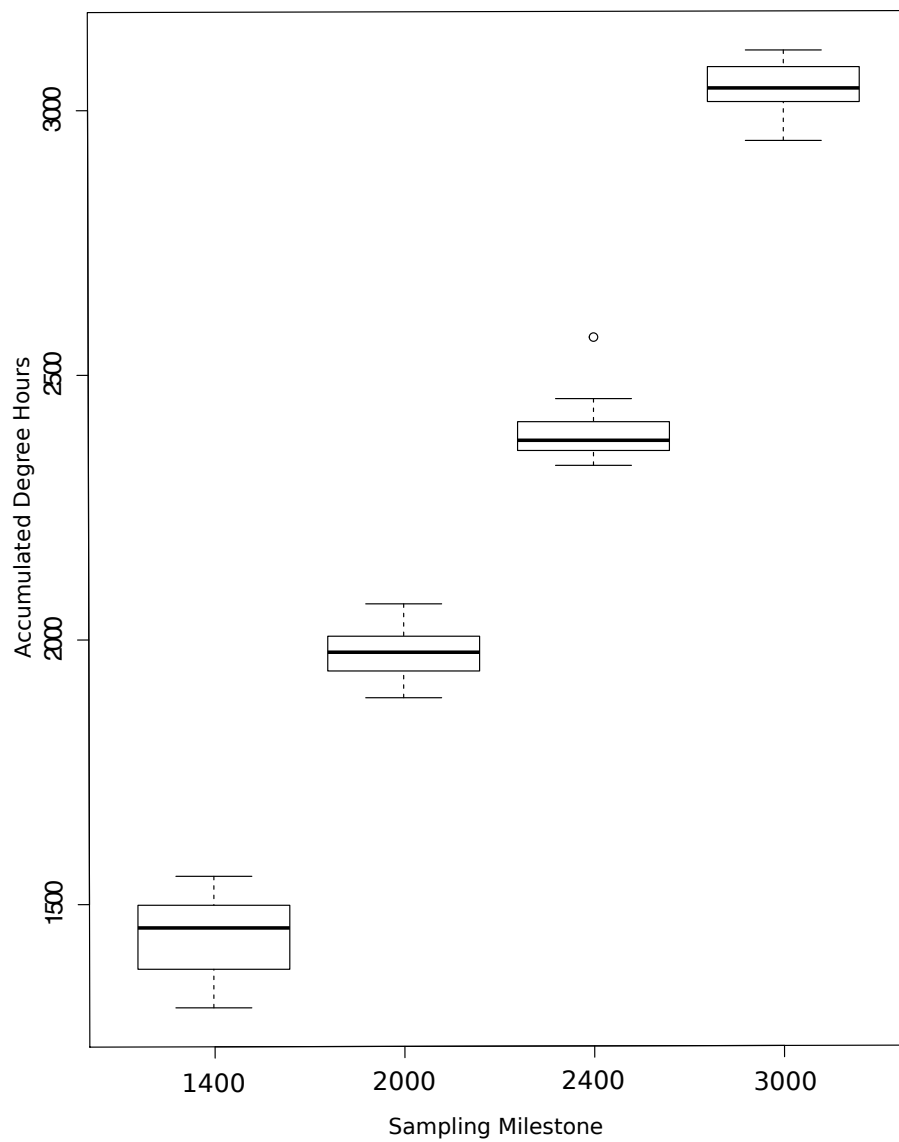


Figure 2.1: The actual sampling times, shown as a range, for each of the timed sampling milestones, 1400, 2000, 2400 and 3000ADH

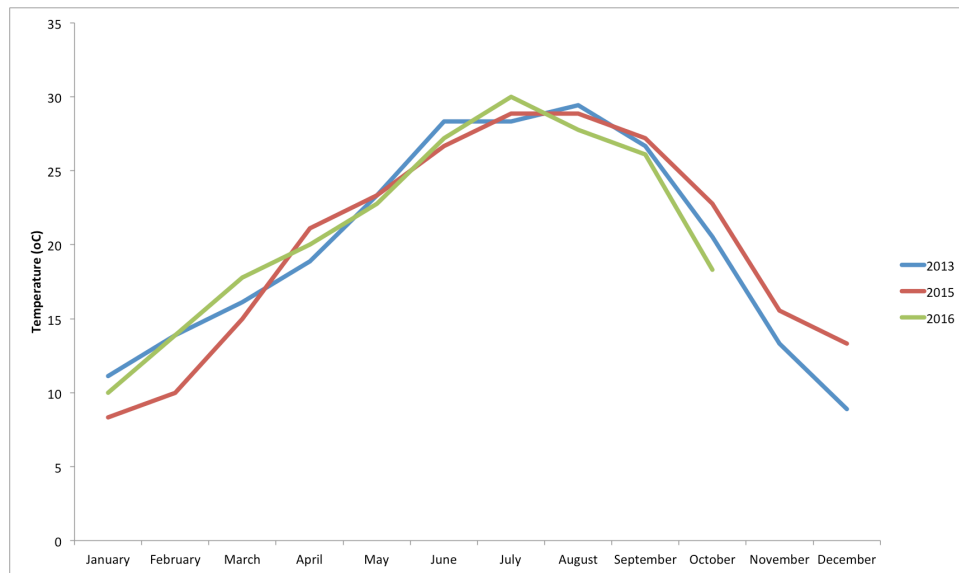


Figure 2.2: Average monthly temperatures in San Marcos, Texas for 2013, 2015 and 2016. Temperatures recorded at the KHYI San Marcos Municipal Airport and collected from Weather Underground (www.wunderground.com)

250ADH outside of the sample milestone resulted in anomalous predictions, which skewed the data. Subsequently, 76 samples across 25 sets of remains were carried forward for analysis in all cases (Tables 2.3,2.4,2.5). First and second instar larvae of this species cannot be identified morphologically with accuracy and so were excluded from the data analysis.

Table 2.3: All samples from field collections at FARF containing larvae of *C. macellaria* in 2013

Donation	Month	Year	ADH Milestone	Actual ADH	Actual TOP
D51	September	2013	1400	1985.30	22-Sep
			2000	2391.04	22-Sep
			2400	2958.31	22-Sep
D53	October	2013	1400	1485.31	11-Oct
			2000	1870.84	11-Oct
			2400	2358.88	11-Oct
			3000	1508.65	11-Oct
D54	October	2013	2000	2328.20	11-Oct
			2400	2670.20	11-Oct
			3000	1312.83	11-Oct

Table 2.4: All samples from field collections at FARF containing larvae of *C. macellaria* in 2015

Donation	Month	Year	ADH Milestone	Actual ADH	Actual TOP
D51	September	2013	1400	1985.30	22-Sep
			2000	2391.04	22-Sep
			2400	2958.31	22-Sep
D53	October	2013	1400	1485.31	11-Oct
			2000	1870.84	11-Oct
			2400	2358.88	11-Oct
			3000	1508.65	11-Oct
D54	October	2013	2000	2328.20	11-Oct
			2400	2670.20	11-Oct
			3000	1312.83	11-Oct
D23	June	2015	1400	2012.86	8-Jun
			2000	2454.27	8-Jun

Table 2.4 – *Continued*

Donation	Month	Year	ADH Milestone	Actual ADH	Actual TOP
D24	June	2015	2400	2942.08	8-Jun
			3000	1942.48	8-Jun
			1400	1480.29	8-Jun
			2000	2066.488	8-Jun
			2400	2405.00	8-Jun
D25	June	2015	3000	1474.98	8-Jun
			1400	1902.76	8-Jun
			2000	2352.94	8-Jun
			2400	1411.12	8-Jun
			1400	1973.90	5-Jun
D27	June	2015	2000	2347.57	5-Jun
			2400	3015.55	5-Jun
			3000	1454.48	5-Jun
			1400	2013.82	4-Jun
D28	June	2015	2000	1458.79	4-Jun
			2400	1371.52	4-Jun
			1400	1870.68	8-Jun
D30	June	2015	2000	2226.34	8-Jun
			2400	3081.57	8-Jun
			1400	1303.83	2-Jul
D35	July	2015	2000	1380.63	2-Jul
			2400	1889.11	2-Jul
			3000	2353.69	2-Jul
			1400	3050.76	15-Jul
D39	July	2015	2000	1443.35	15-Jul
			2400	1889.02	15-Jul
			2000	2158.20	5-Aug
D43	August	2015	1400	2910.05	12-Aug
			2000	1293.59	12-Aug
			2400	2020.38	12-Aug
D45	September	2015	1400	2440.19	28-Aug
			2000	2890.26	28-Aug
			2400	1432.79	28-Aug
D51	September	2015	2000	1936.85	18-Sep
			2400	2375.51	18-Sep
D53	October	2015	1400	2005.33	2-Oct
			2400	2376.59	2-Oct
D54	October	2015	1400	1551.70	2-Oct
			2000	2005.35	2-Oct

Table 2.4 – *Continued*

Donation	Month	Year	ADH Milestone	Actual ADH	Actual TOP
D61	November	2015	2400	2402.95	2-Oct
			3000	3113.02	2-Oct
			1400	1476.11	6-Nov
			2000	2570.49	6-Nov
			2400	1978.81	6-Nov
			3000	2370.62	6-Nov

Table 2.5: All samples from field collection containing larvae of *C. macellaria* in 2016

Donation	Month	Year	ADH Milestone	Actual ADH	Actual TOP
D06	March	2016	2000	2005.33	19-Feb
			2400	2376.59	19-Feb
D08	March	2016	1400	1380.03	29-Feb
			2000	1889.11	29-Feb
			2400	2353.69	29-Feb
			3000	3050.76	29-Feb
D26	June	2016	1400	1293.59	21-Jun
			2000	2020.38	21-Jun
			2400	2440.19	21-Jun
			3000	2890.26	21-Jun
D30	June	2016	1400	1303.83	20-Jun
D67	February	2016	1400	1454.48	26-Jan
			2000	2013.82	26-Jan
			2400	2365.40	26-Jan
D23	June	2016	1400	1371.52	16-Jun
			2000	1870.68	16-Jun
			2400	2226.34	16-Jun
			3000	3081.57	16-Jun
D28	June	2016	1400	1338.06	21-Jun

2.3.3 Estimations by larval development stage

Boatright and Tomberlin

Time of placement for thirteen of the 25 sets of remains, covering 13 out of 76 total samples were accurately predicted using mean predictions of stage across all temperature treatments (Appendix 1, Tables A.4,A.5,A.6). All the accurate predictions were in the 1400ADH milestone, with no accurate predictions in any of the other milestones. The Pork 28.2°C treatment performed best overall, with 13 correct predictions. Pork 20.8°C and Pork 24.3°C performed the worst with only two accurate predictions each. All Details of minimum and maximum stage predictions can be found in Appendix 1, Tables A.1, A.2, A.2 and Tables A.7,A.8,A.9, respectively.

Byrd and Butler

Time of placement for twenty one out of the 25 sets of remains, covering 27 out of 76 total samples were accurately predicted using mean predictions of stage across all temperature treatments (Appendix 1, Tables A.10,A.11, A.12. Accurate predictions were in the 1400ADH and 200ADH milestones only. Each treatment performed about equally well, 25°C was the best with fourteen accurate predictions, 15.6°C, 21.1°C and 32.2°C predicted thirteen actual TOPs each and 26.7°C accurately predicted eleven.

Analysis by two-way ANOVA

The percent ADH coverage data for all temperature treatments from both publications were analysed using a two-way ANOVA ($p < 0.05$), separated by milestone, with a Tukey's HSD *post hoc* test. At the 1400 milestone (Table 2.7), three treatments: Byrd and Butler, 24.3°C and 26.7°C and Boatright and Tomberlin 28.2° had means closest to 100 (actual range 104.77 - 106.45), making them the most accurate predictors (i.e. the most accurate percent coverage of the actual TOP of the remains). At the 2000 milestone Table 2.8), the highest mean and closest value to the actual TOP was Byrd and Butler's 15.6°C

Table 2.6: Summary of accurate predictions, milestone and seasonal coverage made by data from Boatright and Tomberlin and Byrd and Butler across all phenotypes

Phenotype	Data source	No. remains	No. samples	Milestones	Seasons
Stage	Boatright and Tomberlin	19	20	1400, 2000	Autumn, Spring, Summer
	Byrd and Butler	21	27	1400, 2000	Autumn, Spring, Summer
Length	Boatright and Tomberlin	12	14	1400, 2000	Autumn, Spring, Summer
	Byrd and Butler	21	35	1400, 2000, 2400	Autumn, Spring, Summer
Weight	Boatright and Tomberlin	3	3	1400, 2000	Autumn, Summer

treatment, covering on average 97.03 % of the actual TOP. All other treatments performed poorly, covering only 59.03 - 77.41 % of the actual TOP. Similarly at the 2400 milestone Table 2.9), Byrd and Butler's 15.6°C was the highest mean at only 80.25 % coverage, with all other treatments being significantly lower. Finally at the 3000 milestone Table 2.10), all treatments performed poorly, again, with Byrd and Butler's 15.6°C having the highest mean of only 63.28 % of the total coverage all other treatments being significantly lower.

2.3.4 Estimations by larval length

Lengths of all *C. macellaria* larvae in each of the samples were averaged with 90% confidence intervals (± 1 SD). An initial test using 95% confidence intervals returned a large number of data points that lay outside of the scope of the graph, therefore it was deemed appropriate to reduce the width of the interval. The minimum and maximum values of the confidence intervals were used to report a predicted TOP range estimate using the graphs from both published datasets. Where a range was reported from the graph (e.g., the line hit two points on the graph), the minimum TOP estimate from the lower confidence interval and the maximum TOP estimate from the upper confidence interval was used. The range is reported in full for the mean value as a mean cannot be taken in this case. As for stage, percent (%) coverage was calculated and a two way ANOVA was run.

Boatright and Tomberlin

When using mean length as the phenotype, predictions for Boatright and Tomberlin

were only able to successfully predict one sample in the 1400ADH milestone in 2013 (Appendix 2, Tables B.4). However, minimum estimates (Appendix 2, Tables B.1,B.2,B.3) were able to successfully predict eleven out of 25 sets of remains, covering thirteen out of 76 total possible samples across all temperature treatments. Ten of the accurate predictions were in the 1400ADH milestone and three in the 2000ADH milestone, with no accurate predictions in the 2400ADH and 3000ADH milestones. Each temperature treatment performed about equally with Pork/Equine 20.8°C making six accurate predictions, Pork/Equine 24.3°C making five and Pork/Equine 28.2°C making four. All Details of mean and maximum length predictions can be found in Appendix 2, Tables B.4,B.5,B.5 and Tables B.7,B.8,B.9, respectively.

Byrd and Butler

Time of placement for twenty one out of the 25 sets of remains, covering 31 out of 76 total samples were accurately predicted using mean predictions of length across all temperature treatments (Appendix 1, Tables B.13,B.14,B.15). Fourteen of the successful predictions were in the 1400ADH milestone, fifteen were in the 2000ADH milestone and two were in the 2400ADH milestone, with none in the 3000ADH milestone. The maximum number of correct predictions, seventeen, were made by the 32.2°C treatment and the lowest was nine predictions made in the 26.7°C treatment. All Details of minimum and maximum length predictions can be found in Appendix 2, Tables B.10, B.11, B.11 and Tables B.16,B.17,B.18, respectively.

Analysis by two-way ANOVA

All treatments from both publications were analysed using a two-way ANOVA as described in the previous section ($p < 0.05$). At the 1400 milestone (Table 2.7), Byrd and Butler 32.2°C, 26.7°C, 25°C and 21.1°C and made the most accurate predictions (between 92.05 - 107.42 % total coverage) with no significant differences between these treatments. Similarly, at the 2000 ADH milestone (Table 2.8), the same four treatments were the best

predictors in the model, however the range of coverage was only 81.13 - 90.33 %. In the 2400 milestone (Table 2.9), Byrd and Butler 32.2°C, 26.7°C and 21.1°C gave the best coverage (between 83.66 - 93.44 %). Finally, the 3000 milestone (Table 2.10) the best predictors were again Byrd and Butler 32.2°C, 26.7°C, 25°C and 21.1°C, making between 92.72 - 133.33 % coverage).

2.3.5 Estimations by larval weight

Weights of all *C. macellaria* larvae in each of the samples were averaged with 90% confidence intervals (± 1 SD) intervals to test the accuracy of published weight data in Boatright and Tomberlin[49]. The minimum and maximum values of the confidence intervals were used to report a TOP range estimate using the graphs from both published datasets. Where a range was reported from the graph, the minimum TOP estimate from the lower confidence interval and the maximum TOP estimate from the upper confidence interval was used. All the ranges were converted to deviations from the actual TOP for further analysis.

Boatright and Tomberlin

When using mean weight as the phenotype of interest, only a single sample was accurately predicted in the 1400ADH milestone in 2016 (Appendix 3, Table C.5). Using minimum weight instead (Appendix 3, Tables C.1,C.2,C.3, accurate predictions were made for five out of 25 sets of remains, covering five out of 76 total samples. Three predictions were in the 1400ADH milestone group and two were in the 2000ADH milestone group, with no accurate predictions in the other milestone groups. The temperature treatment 24.3°C performed the best in this data, making 4 accurate predictions. All Details of mean and maximum weight predictions can be found in Appendix 3, Tables C.4,C.5,C.5 and Tables C.7,C.8,C.9, respectively.

Table 2.7: Summary of ANOVAs (BodyID and Treatment) for mean stage and length at the 1400 Milestone

Phenotype	Dataset	Model	Source	DF	Mean Square	F ratio	Prob. > F
Stage (time to 3rd instar)	Boatright and Tomberlin	Two-way ANOVA	Model	21	501.031	41.59	<0.0001
			Error	104	12.044		
			Total	125			
		Effects Tests	Body ID	16		13.35	<0.0001
			Treatment	5		131.97	<0.0001
	Byrd and Butler	Two-way ANOVA	Model	20	1684.76	100.19	<0.0001
			Error	84	16.81		
			Total	104			
		Effects Tests	Body ID	16	2737.19	10.17	<0.0001
			Treatment	4	30958.07	460.29	<0.0001
	All	Two-way ANOVA	Model	26	2014.26	153.05	<0.0001
			Error	204	13.16		
			Total	230			
		Effects Tests	Body ID	16	5291.43	25.13	<0.0001
			Treatment	10	47079.26	357.74	<0.0001
Length	Boatright and Tomberlin	Two-way ANOVA	Model	18	674.27	11.47	<0.0001
			Error	44	58.77		
			Total	62			
		Effects Tests	Body ID	16	1911.9	2.03	0.0322
			Treatment	2	10224.96	86.99	<0.0001
	Byrd and Butler	Two-way ANOVA	Model	20	2517.1	6.85	<0.0001
			Error	84	367.5		
			Total	104			
		Effects Tests	Body ID	16	41973.26	7.13	<0.0001
			Treatment	4	8368.71	5.69	0.0004
	All	Two-way ANOVA	Model	23	4799.84	13.29	<0.0001
			Error	144	360.94		
			Total	167			
		Effects Tests	Body ID	16	25365.69	4.39	<0.0001
			Treatment	7	85030.57	33.65	<0.0001

Table 2.8: Summary of ANOVAs (BodyID and Treatment) for mean stage and length at the 2000 Milestone

Phenotype	Dataset	Model	Source	DF	Mean Square	F ratio	Prob. > F
Stage (time to 3rd instar)	Boatright and Tomberlin	Two-way ANOVA	Model	23	208.02	229.24	<0.0001
			Error	108	0.907		
			Total	131			
			Body ID	18	356.25	21.81	<0.0001
			Treatment	5	4428.22	976.01	<0.0001
	Byrd and Butler	Two-way ANOVA	Model	22	801.26	612.33	<0.0001
			Error	87	1.309		
			Total	109			
			Body ID	18	378.8	16.08	<0.0001
			Treatment	4	17248.93	3295.45	<0.0001
	All	Two-way ANOVA	Model	28	962.97	955.95	<0.0001
			Error	213	1.007		
			Total	241			
			Body ID	18	732.34	40.38	<0.0001
			Treatment	10	26230.83	2603.98	<0.0001
Length	Boatright and Tomberlin	Two-way ANOVA	Model	20	345.13	1.72	0.0642
			Error	45	199.67		
			Total	65			
			Body ID	18	2848.83	0.79	0.6976
			Treatment	2	4053.81	10.15	0.0002
	Byrd and Butler	Two-way ANOVA	Model	22	3184.43	14.7634	<0.0001
			Error	87	215.7		
			Total	109			
			Body ID	18	57379.33	14.77	<0.0001
			Treatment	4	12678.16	14.69	<0.0001
	All	Two-way ANOVA	Model	25	5712.61	18.95	<0.0001
			Error	150	301.37		
			Total	175			
			Body ID	18	42774.62	7.88	<0.0001
			Treatment	7	100040.59	47.422	<0.0001

Table 2.9: Summary of ANOVAs (BodyID and Treatment) for mean stage and length at the 2400 Milestone

Phenotype	Dataset	Model	Source	DF	Mean Square	F ratio	Prob. > F
Stage (time to 3rd instar)	Boatright and Tomberlin	Two-way ANOVA	Model	22	147.15	63.15	<0.0001
			Error	109	2.33		
			Total	131			
		Effects Tests	Body ID	17	205.95	5.19	<0.0001
			Treatment	5	3031.5	260.19	<0.0001
	Byrd and Butler	Two-way ANOVA	Model	21	572.68	180.14	<0.0001
			Error	88	3.17		
			Total	109			
		Effects Tests	Body ID	17	219.06	4.05	<0.0001
			Treatment	4	11807.34	928.54	<0.0001
	All	Two-way ANOVA	Model	27	680.72	272.13	<0.0001
			Error	214	2.5		
			Total	241			
		Effects Tests	Body ID	17	423.44	9.95	<0.0001
			Treatment	10	17956.12	717.82	<0.0001
Length	Boatright and Tomberlin	Two-way ANOVA	Model	19	231.13	4.11	<0.0001
			Error	46	56.21		
			Total	65			
		Effects Tests	Body ID	17	1889.99	1.97	0.0342
			Treatment	2	2501.64	22.24	<0.0001
	Byrd and Butler	Two-way ANOVA	Model	21	3424.17	20.84	<0.0001
			Error	88	164.29		
			Total	109			
		Effects Tests	Body ID	17	61373.64	21.97	<0.0001
			Treatment	4	10533.93	16.02	<0.0001
	All	Two-way ANOVA	Model	24	6409.19	25.43	<0.0001
			Error	151	252.02		
			Total	175			
		Effects Tests	Body ID	17	42242.18	9.86	<0.0001
			Treatment	7	111568.44	63.24	<0.0001

Table 2.10: Summary of ANOVAs (BodyID and Treatment) for mean stage and length at the 3000 Milestone

Phenotype	Dataset	Model	Source	DF	Mean Square	F ratio	Prob. > F
Stage (time to 3rd instar)	Boatright and Tomberlin	Two-way ANOVA	Model	13	77.04	320.44	<0.0001
			Error	52	0.24		
			Total	65			
	Effects Tests		Body ID	8	65.56	34.08	<0.0001
			Treatment	5	936.04	778.61	<0.0001
	Byrd and Butler	Two-way ANOVA	Model	12	309.62	868.73	<0.0001
			Error	42	0.356		
			Total	54			
	Effects Tests		Body ID	8	69.76	24.46	<0.0001
			Treatment	4	3645.76	2557.26	<0.0001
Length	All	Two-way ANOVA	Model	18	315.49	1150.28	<0.0001
			Error	102	0.274		
			Total	120			
	Effects Tests		Body ID	8	134.82	61.44	<0.0001
			Treatment	10	5544.07	2021.36	<0.0001
	Boatright and Tomberlin	Two-way ANOVA	Model	10	150.09	40.07	<0.0001
			Error	22	3.746		
			Total	32			
	Effects Tests		Body ID	8	171.55	5.72	0.0005
			Treatment	2	1329.36	177.45	<0.0001
Length	Byrd and Butler	Two-way ANOVA	Model	12	3396.3	9.97	<0.0001
			Error	42	340.61		
			Total	54			
	Effects Tests		Body ID	8	34716.71	12.74	<0.0001
			Treatment	4	6038	4.43	0.0044
	All	Two-way ANOVA	Model	15	8527.54	22.75	<0.0001
			Error	72	374.71		
			Total	87			
	Effects Tests		Body ID	8	22298.47	7.43	<0.0001
			Treatment	7	105615.62	40.26	<0.0001

Table 2.1.1: Summary of ANOVAs (BodyID and Treatment) for mean weight by milestone

Milestone	Dataset	Model	Source	DF	Mean Square	F ratio	Prob. > F
1400	Boatright and Tomberlin	Two-way ANOVA	Model	18	373.68	4.89	<0.0001
			Error	44	76.36		
			Total	62			
		Effects Tests	Body ID	16	4500.54	3.68	0.0003
			Treatment	2	2225.82	14.57	<0.0001
2000	Boatright and Tomberlin	Two-way ANOVA	Model	20	440.62	4.73	<0.0001
			Error	45	93.08		
			Total	65			
		Effects Tests	Body ID	18	7969.12	4.75	<0.0001
			Treatment	2	843.37	4.53	0.0161
2400	Boatright and Tomberlin	Two-way ANOVA	Model	19	252.39	2.38	0.0083
			Error	46	105.83		
			Total	65			
		Effects Tests	Body ID	17	4332.37	2.4079	0.0093
			Treatment	2	463.08	2.1877	0.1237
3000	Boatright and Tomberlin	Two-way ANOVA	Model	19	252.39	2.38	0.0083
			Error	46	105.83		
			Total	65			
		Effects Tests	Body ID	17	4332.37	2.4	0.0093
			Treatment	2	463.08	2.18	0.1237

Analysis by two-way ANOVA

Results from the two-way ANOVA ($p < 0.05$) at the 1400 milestone (Table 2.11) found that the 24.8°C treatment had the highest coverage, but only with 73.28 % coverage. This model performed even worse at the 2000 milestone (Table 2.11), with the highest coverage being made by the same treatment, but with only 51.51 % coverage. At the 2400 milestone (Table 2.11), there were no significant differences between treatments and a coverage of only 37.11 - 43.34 %. Finally, for the 3000 milestone (Table 2.11), the 24.8°C treatment again had the highest coverage, but again it was very low at just 36.54 %.

2.3.6 Effect of season

Boatright and Tomberlin

Applying stage (minimum, mean and maximum) phenotype data resulted in correct predictions for seven months across three seasons. Twelve samples being from summer (June-August), six from autumn (September-November), and two from spring (March).

Applying length (minimum, mean and maximum) phenotype data resulted in correct predictions for six months across three seasons. Eight samples being from summer (June-August), five from autumn (September-October), and one from spring (March).

Applying weight (minimum, mean and maximum) phenotype data resulted in correct predictions for two months across two seasons. Two samples being from summer (June), and one from autumn (October).

Byrd and Butler

Applying stage (minimum, mean and maximum) phenotype data resulted in correct predictions for seven months across three seasons. Nineteen samples being from summer (June-August), eight from autumn (September-November), and one from spring (March).

Applying length (minimum, mean and maximum) phenotype data resulted in correct predictions for seven months across three seasons. Twenty three samples being from

summer (June-August), ten from autumn (September-November), and two from spring (March).

Analysis by two-way ANOVA

For the stage phenotype, at 1400 ADH (Table 2.12), no interaction effect or effect of season, with treatment accounting for most of the variation in the data ($F_{10} = 38.61$, $p < 0.0001$). Likewise, this was the case for 3000ADH (Table 2.15) (season: $F_3 = 1.69$, $p = 0.1902$; treatment: $F_{10} = 178.73$, $p < 0.0001$). However, at 2000ADH (Table 2.13) there was a significant effect of season ($F_3 = 11.75$, $p < 0.0001$) and treatment ($F_{10} = 271.13$, $p < 0.0001$), but no season x treatment effect. Similarly, there was an effect of season at 2400ADH (Table 2.14), although less significant ($F_3 = 2.92$, $p = 0.0348$), again with a significant effect of treatment ($F_{10} = 164.5$, $p < 0.0001$).

Similar patterns were observed when considering the length phenotype. At 1400 ADH (Table 2.12) and 3000ADH (Table 2.15) only treatment was significant ($F_{10} = 38.61$, $p < 0.0001$; $F_{10} = 178.73$, $p < 0.0001$, respectively). At 2000ADH (Table 2.13) treatment ($F_{10} = 271.13$, $p < 0.0001$) and season ($F_3 = 11.75$, $p < 0.0001$) were both very significant, and at 2400ADH (Table 2.14), treatment ($F_{10} = 164.5$, $p < 0.0001$) was highly significant and season was less significant ($F_3 = 2.92$, $p = 0.0348$).

When using weight as phenotype, there were variable effects of season across the different milestone groups; there are also similar groupings to the length and phenotype data. At the 1400 ADH (Table 2.12) and 3000ADH (Table 2.15), the ANOVA showed no significant differences between means ($F_{11} = 2.01$, $p = 0.045$; $F_{11} = 2.11$, $p = 0.0753$, respectively). Conversely, at the 2000ADH milestone (Table 2.13), there was a significant effect of season ($F_3 = 9.74$, $p < 0.0001$), but also a significant season x treatment interaction effect ($F_6 = 6.26$, $p < 0.0001$). At 2400ADH (Table 2.14), there was also a significant effect of treatment ($F_2 = 12.74$, $p < 0.0001$) and a significant season x treatment interaction effect ($F_6 = 12.08$, $p < 0.0001$).

Table 2.12: Summary of ANOVAs (Season and Treatment) for all samples at the 1400 Milestone

Phenotype	Dataset	Model	Source	DF	Mean Square	F ratio	Prob. > F
Stage	All	Full factorial ANOVA	Model	43	1098.86	26.33	<0.0001
			Error	187	41.74		
			Total	230			
		Effects test	Season	3	167.98	1.34	0.2622
			Treatment	10	16115.27	38.61	<0.0001
			Season x Treatment	30	3.61	0.00	1.0000
Length	All	Full factorial ANOVA	Model	43	1098.86	26.32	<0.0001
			Error	187	41.74		
			Total	230			
		Effects test	Season	3	167.98	1.34	0.2622
			Treatment	10	16115.27	38.61	<0.0001
			Season x Treatment	30	3.61	0.0029	1.0000
Weight	Boatright and Tomberlin	Full factorial ANOVA	Model	11	278.67	2.02	0.0450
			Error	51	137.67		
			Total	62			
		Effects test	Season	3	823.08	1.99	0.1267
			Treatment	2	778.99	2.83	0.0684
			Season x Treatment	6	16.51	0.02	1.0000

Table 2.13: Summary of ANOVAs (Season and Treatment) for all samples at the 2000 Milestone

Phenotype	Dataset	Model	Source	DF	Mean Square	F ratio	Prob. > F
Stage	All	Full factorial ANOVA	Model	43	613.41	151.60	<0.0001
			Error	198	4.05		
			Total	241			
		Effects test	Season	3	142.70	11.75	<0.0001
			Treatment	10	10970.82	271.13	<0.0001
Length	All	Full factorial ANOVA	Season x Treatment	30	3.05	0.03	1.00
			Model	43	613.4	151.6	<0.0001
			Error	198	4.046		
		Effects test	Total	241			
			Season	3	142.7	11.75	<0.0001
Weight	Boatright and Tomberlin	Full factorial ANOVA	Treatment	10	10970.82	271.13	<0.0001
			Season x Treatment	30	3.054	0.02	1.00
			Model	11	687.85	6.83	<0.0001
		Effects test	Error	54	100.65		
			Total	65			
			Season	3	2940.32	9.74	<0.0001
			Treatment	2	1236.94	6.15	0.0039
			Season x Treatment	6	3782.63	6.26	<0.0001

Table 2.14: Summary of ANOVAs (Season and Treatment) for all samples at the 2400 Milestone

Phenotype	Dataset	Model	Source	DF	Mean Square	F ratio	Prob. > F
Stage	All	Full factorial ANOVA	Model	43	418.55	90.35	<0.0001
			Error	198	4.63		
			Total	241			
		Effects test	Season	3	40.71	2.92	0.0348
			Treatment	10	7620.23	164.50	<0.0001
Length	All	Full factorial ANOVA	Season x Treatment	30	0.86	0.01	1
			Model	43	418.551	90.35	<0.0001
			Error	198	4.63		
		Effects test	Total	241			
			Season	3	40.71	2.92	0.0348
Weight	Boatright and Tomberlin	Full factorial ANOVA	Treatment	10	7620.96	164.50	<0.0001
			Season x Treatment	30	0.86	0.0062	1.00
			Model	11	566.97	8.93	<0.0001
		Effects test	Error	54	63.47		
			Total	65			
			Season	3	1173.86	6.17	0.0011
			Treatment	2	1617.19	12.74	<0.0001
			Season x Treatment	6	4599.76	12.08	<0.0001

Table 2.15: Summary of ANOVAs (Season and Treatment) for all samples at the 3000 Milestone

Phenotype	Dataset	Model	Source	DF	Mean Square	F ratio	Prob. > F
Stage	All	Full factorial ANOVA	Model	32	173.44	97.43	<0.0001
			Error	88	1.78		
			Total	120			
		Effects test	Season	2	6.02	1.69	0.1902
			Treatment	10	3181.63	178.73	<0.0001
Length	All	Full factorial ANOVA	Season x Treatment	20	0.13	0.00	1.00
			Model	32	173.44	97.43	<0.0001
			Error	88	1.78		
		Effects test	Total	120			
			Season	2	6.021	1.69	0.1902
Weight	Boatright and Tomberlin	Full factorial ANOVA	Treatment	10	3181.63	178.73	<0.0001
			Season x Treatment	20	0.13	0.0037	1.00
			Model	8	40.59	2.11	0.0753
		Effects test	Error	24	19.24		
			Total	32			
		Effects test	Season	2	23.33	0.61	0.5535
			Treatment	2	179.89	4.68	0.0193
			Season x Treatment	4	6.30	0.08	0.9872

2.4 Discussion

The results indicate that the data relating to the stage phenotype was able to make the most accurate predictions in both datasets when compared with the length and weight phenotype data. However, length phenotype data published by Byrd and Butler made the highest number of accurate predictions (35) overall, making this the best dataset to use in this study. This lends support to the current casework practice of using stage and also highlights that length and weight estimations are less accurate and should be used with caution in legal proceedings. However, it is very important to consider the statistical and real-world significance of the results separately. For example, although Byrd and Butler stage data performed well statistically in this study, it was still only able to accurately predict TOP in 27 out of 76 samples. If we were to consider this in terms of 76 real cases, the ability of this data to make accurate predictions is still worryingly low.

There were significant differences in the accuracy of predictions made across the four sampling milestones as seen in the percent coverage data (Appendices 1-3). The accuracy of TOP predictions decreased across the timeline of the sampling milestones. Overall, there were more accurate predictions in the earlier milestones (1400 and 2000 ADH) than in the later milestones (2400 and 3000 ADH). This could pose a serious difficulty in terms of casework, particularly during the summer months when 2400ADH can be reached within a matter of one or two days; samples taken from older remains could produce more inaccurate estimates, when using the current datasets.

The Byrd and Butler data had consistently slower development rates than the Boatrigh and Tomberlin data and was able to accurately predict a higher number of cases across all three phenotypes tested (Table 2.6). This is particularly interesting as the Boatrigh and Tomberlin data was derived from a Texas population of *C. macellaria* and the Byrd and Butler data was derived from a Florida population. Phenotypic variation among

populations in Texas and Florida could also account for some of the differences found in development times and subsequent prediction accuracy. Although Texas and Florida share a similar climate, the geographical distance alone could account for population differences, as well as any number of local selection biases or genetic drift. The work of Tarone and Foran [91] confirms that genetic differences exist in local blow fly populations and this could go some way towards explaining why there are differences between not only the Texas and Florida populations, but also the subpopulations found between College Station, Texas [49] and San Marcos, Texas (the current study). This may also lend some credence to the ability of experiments to reduce error when they are appropriately designed.

As prediction accuracy was low in many cases in the current study we can safely assume that there are other factors affecting development in the field that are not being accounted for in our predictions; this study was limited to addressing ambient temperature only. A number of factors are highlighted in the literature pertaining to blow fly development, for example, photoperiod [151], maggot mass temperature [152], humidity [153] and diet type [77] and diet condition (i.e. decomposed, ground, whole etc.) [75]. Each of these factors (and possibly others) will have an impact on the ability of laboratory data to reflect field conditions and therefore the ability of the subsequent degree hour models to accurately predict development rates.

In the current study, an important factor affecting the ability of the datasets to predict the actual TOP (and a cause of rate summation effect in ADH models in general) is the developmental differences observed between constant and fluctuating temperatures. The Byrd and Butler dataset included a constant rearing temperature and four temperatures fluctuating around a mean whereas the Boatright and Tomberlin data used constant rearing temperatures. This may go some way towards explaining why the Byrd and Butler data performed better overall, however it is important to note that there was no difference in

the ability of the constant temperature treatment in Byrd and Butler to accurately predict the actual TOP when compared to the other treatments. The effects of fluctuating vs. constant temperatures on blow flies has been shown to be variable in the literature. Other than the Byrd and Butler development dataset evaluated in this study, there have been no further investigations on the effect of fluctuating temperature on *C. macellaria*. However, in a study of four forensically important blowflies, Niederegger et al. [146] found that fluctuating temperatures caused faster development in (*Meigen*) (Diptera: Sarcophagidae) and *Lucilia illustris* (Diptera: Calliphoridae) but caused slower development in *Calliphora vicina* and *Calliphora vomitoria* (Diptera: Calliphoridae). The species specific nature of the influence of fluctuating temperature, therefore, permits further work to be done to examine it's effect on *C. macellaria*.

Irregular sample sizes across the field samples and various data groupings are likely to have had an effect on the weighting in statistical analysis. Samples contained differing numbers of larvae, so weight and length averages may have been somewhat skewed. By-will body donation sites are unable to control the flow of remains into the facility and therefore studies such as this will almost always suffer differential sample sizes across the course of a year, a difficulty not faced by researchers using swine analogs. In the current study, each set of remains (and each of the samples taken from each of the bodies) should be considered a separate experimental unit as no true replication took place. The ANOVAs including Body ID as a factor (Tables 2.7 - 2.11) revealed a significant effect in many cases, indicating variation between bodies. Moreover, their arbitrary grouping for statistical analysis should be read with the caveat that this is likely an act of pseudoreplication [25]. Likewise, the number of larvae in each sample could be controlled for, with exception to cases where there are only a small number of larvae present. The protocol for this study did call for at least five of each different type of larvae that could be seen, but in practice, there are real constraints on the ability of the experimenters to be able to ensure

larvae are the same by sight alone, unless the larvae have characteristics that are very easy to spot.

In summary, my hypothesis regarding the limited use of the existing development datasets was accepted, particularly when considering the length and weight phenotypes. Stage shows the most promise in terms of accuracy across each dataset, however this accuracy decreased as the sampling milestone became further removed temporally from the actual TOP. Additionally, my hypothesis that there would be an effect of season on the ability of the datasets to make accurate predictions was only true in some cases and the some of the confounding factors discussed (such as pseudoreplication) may have reduced the inferential power of the analysis.

Several factors have been discussed here that may affect the usefulness of laboratory development data, as well as highlighting the limitations of the current study and problems for future fieldwork to overcome. My hope is that the forensic entomology community will find ways to incorporate these findings as a tool for designing future developmental studies and that validation and evaluation will become a core part of standard practice for future publications.

3. SUMMARY AND SUGGESTIONS FOR FUTURE WORK

To my knowledge, this study is the first large scale blind field evaluation of forensic entomology datasets for the secondary screwworm *Cochliomyia macellaria* (Diptera: Calliphoridae) using human remains to estimate time of colonisation (TOC) as related to time of placement (TOP). As such, it offers a unique insight into the processes and possible challenges that lay ahead for future studies of this kind; in particular the way in which data are collected from the field and applied to forensic investigations. Moreover, by evaluating existing development datasets, it is easier to highlight issues with their associated designs and presentation for use in forensic casework. In the current study I demonstrated that there were some serious difficulties in the ability of laboratory development data to accurately predict the time of placement (TOP) of human remains, which highlights the significance of TOP vs. TOC and how they relate to one another. This is not a surprising outcome given previous VanLaerhoven's [92] evaluation study, but should be cause for concern. Moreover this study demonstrated a possible effect of season on the ability of using the existing data to predict TOP, suggesting that more attention should be paid to the complexity of environmental and genotypic effects on blow fly development.

Development data reported without a range clearly are less likely to yield quick and accurate results of TOP. For example, when using the Byrd and Butler [51] stage data, only a single point estimate for TOP could be made using the means presented in the publication. Some practitioners might be tempted to create their own confidence intervals, which I did for the length data. However this assumption could introduce twofold error; the confidence intervals would need to be calculated by hand and the subsequent calculations may lie well outside of the actual biological range of the organism. When I initially made predictions on the length data, 95% confidence intervals were calculated,

but this returned 50 predictions that were outside of the possible length of real larvae that it became necessary to reduce the confidence intervals to 90%. Introducing error such as this when assessing evidence is dangerous when victims, suspects and their families are considered. Furthermore, it means that when data are not presented in a meaningful way, the hard work and expense of the experimenter become moot as their product does not perform and will become quickly irrelevant. A similar conclusion about point estimates was drawn when evaluating development data for the black soldier fly, *Hermetia illuciens* (L.) (Diptera: Stratiomyidae) (Cuttiford et al., *working manuscript*). All but one of the five datasets evaluated were able to make estimations with any degree of accuracy, and those that performed the worst were those reporting only a mean or minimum development rate. Granted, development data pertaining to black soldier flies is not necessarily geared towards use in the forensic sciences as this species is traditionally an agricultural waste decomposer, however the point still parallels the current study. One of the reasons that the Boatright and Tomberlin [49] data are consistently faster than the Byrd and Butler [51] data is that it only reports minimum development times \pm standard deviation and is therefore also not a true minimum, mean and maximum range representation.

The publishing of graphs for length and weight data incurred a huge time sink when carrying out the analysis. Due to the large numbers of predictions that needed to be made, it was necessary to sample the graphs to as fine a point as possible and automate the lookups of each prediction. This approach could mean that the coarse resolution of sampling the hardcopy graphs returned erroneous results. Future publications of length and weight-based development data should look to making raw data or at least detailed tables available. I attempted to present the data accumulated in the current study in ways that made it accessible to practitioners by reporting the data predictions and to make it more meaningful by reporting the percentage coverage, with a view to giving a quick overview of the precision of the estimate overall. Additionally, I included visually meaningful plots,

some that might be considered for use in courtroom, where it was very clear how close or far a particular prediction was from the actual TOP. It would also be beneficial to begin researching ways to make these data more accessible by practitioners. Resources such as the *Forenseek* database (www.forenseek.org) published by forensic entomologists in Europe are a clear step in the right direction. Creating automated (i.e. computer-based) predictions for casework is paramount to upholding scientific rigor in evidential analysis and should not be left up to practitioners to make individual decisions in each casework instance. Making whole data sets available in these cases could reduce error significantly and allows for repeatable, objective results. Moreover, in cases such as the current study, where large numbers of remains are sampled, automating the process makes it faster and more reliable and could prove important for mass death or disaster scenes.

The use of an accurate and biologically meaningful thermal minimum or base temperature was outlined in the literature review. For the current study, I chose the thermal minimum of 10°C, which was used in both of the published datasets for this evaluation. However, on investigation, it became clear that there was no published account for the selection of this temperature as the thermal minimum and its use was a precedent that had been carried forward across the past two decades, possibly based on other flies adapted for hotter climates. Such a trend is worrying in light of courtroom proceedings and it would be worth a future survey to investigate if a similar trend is occurring across the board. This particular assumption is more easily fixed by either choosing an appropriate method of calculating the thermal minimum (such as the x-intercept method outlined in the literature review) or carrying out studies on forensically-important species to investigate their real thermal limits.

Another scenario affecting prediction accuracy of the laboratory data could be competition within maggot masses. Maggot masses on the human remains vary in size, but can sometimes cover large areas and consist of thousands of individual larvae, particu-

larly in the summer months (*personal observation*). These typically consist of communities of differing species, competing and sometimes preying on one another (for example the predatory behaviour of *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) on *C. macellaria* [87]. Competitive interactions are known to have differential effects on development (for example: [80, 85]), with some selection towards resource partitioning [154, 155] . However, for blow flies feeding on an ephemeral resource, fast arrival time and development are key to survival, so without the option of resource partitioning, larvae may experience an offset in development as energy is allocated to competition or even escape from predation.

The Accumulated Degree Hour (ADH) monitor application was successful in allowing colleagues to time their sample collections at each set of remains. Software of this kind will have use for future evaluation work for forensic entomology and may also have application for other types of research using ADH timings. It also proved useful for gathering historical data in a more user-friendly fashion, without the need to search through large agency databases and download many separate files. For this reason alone, consideration should be given to funding research and development of this sort of software for casework and research.

A perennial issue with the morphological identification of forensically important fly species is the paucity of robust keys for larvae. In the current study, I used the Center for Disease Control (CDC) pictorial key for common species of larvae (www.cdc.gov), which is most commonly used in casework in the F.L.I.E.S Facility, Texas A&M University. To my knowledge there is no other comprehensive key for carrion-using blow fly larvae for the southern USA. Phenotypic variation both within and between larval congeners could be responsible for some yet unquantified error when evaluating evidence. Moreover, identification keys for first and second instar larvae are extremely rare and certainly none could be found for *C. macellaria*. The advent of molecular methods for larvae identification is

helpful in some cases, but when dealing with very large numbers (over 3000 individuals were analysed for this study alone), it is simply not feasible and so morphological identification must be relied on. There is a dire need for easy to use, pictorial larval keys for species of forensic importance.

As this study was the first of its kind, it was necessary to set broad parameters on the data collection - such as several sampling points and large numbers of larval samples. It is clear, in this instance at least, that TOP based on samples taken at the later ADH milestones (1400, 2000, 2400 and 3000 ADH) were never accurately predicted by the datasets. Depending on the nature of a study, it might be more beneficial to concentrate on samples closer to the actual TOP to get a finer resolution of how the dataset perform, or to take more samples closer to the actual TOP and fewer samples later on. Certainly, understanding how well a particular dataset performs at various distances from the actual TOP (if we assume that it is the same as TOC) is crucial for casework, but may not be so for other kinds of basic research. Likewise, it may not be necessary to have more than 30 or so larvae per sample when averages are being calculated for estimations. In the current study, there were many samples that included much less than 30 individuals in a sample. However this was largely due to the unknown identification of larvae taken from natural communities on the remains. Future studies would benefit from taking large samples and then randomly selecting a predetermined number of larvae of the same species so that sample sizes do not skew the data.

As discussed in the results, species other than *C. macellaria* were collected from the remains, including large numbers of *Phormia regina* (Meigen) (Diptera: Calliphoridae) and *C. rufifaces*. Future work with the data collected from this study should include the analysis of development datasets for these species. Moreover, attention should be paid to the other species present in smaller numbers such as the *Hydrotaea spp.* and *Lucilia spp.* which were present in a smaller number of samples. These could prove to be of

forensic utility in central Texas and it would be worth investigating their life history and developmental preferences.

There have been a number of publications highlighting inherent problems with experiment design and subsequent statistical inference in forensic entomological fieldwork [19, 25, 150, 156]. Michaud et al. [25] reviewed 63 publications and found that only 17% of them had adequate analysis and design. One of the main points of concern was regarding pseudoreplication, where experimental units are erroneously treated as independent, when they are in fact interdependent either temporally or spatially (or vice versa). A problem with the current study and one that will likely hinder future work with human remains was the lack of control over the timing and placement of remains. As such it was impossible to create true replication over the course of the study; despite some remains being temporally replicated, those remains were placed in different spatial locations. Michaud et al. [25] also discussed the difficulty in preventing cross contamination of larvae, flies and beetles between experimental units, even when more than 50m apart. In the current study some sets of remains were placed less than 1 m apart and therefore independence of this kind may not have been met - although site specific differences do exist. Presenting the data body by body (such as in Appendix 1) is certainly useful, however in order to carry out statistical, samples needed to be grouped. Subsequently, since it is not possible to gauge the degree of interdependence of some of the remains (such as those being placed closely together), statistical inference may have been compromised. Control over timing and placement of remains is certainly a benefit of working with swine analogues, however, it would be encouraging to see more interactivity between anthropology facilities and forensic entomologists to negotiate placements that benefit both parties. The costs associated with the use of donated remains for both parties might be eased if specific funding were put in place by relevant forensic institutions.

In summary, given the focus on more robust frameworks for the forensic sciences and

the call from lawmakers and judges that forensic evidence be held to higher scientific standards [22, 23], future development data should present detailed and informative data that can be understood by jurors and courtroom officials. For example, clear tables, simple explanations of biological parameters and laboratory vs. field effects or plots and figures which make it simple for readers to understand the precision and accuracy of estimations. Difficulties in explaining complex scientific concepts to the layperson are known to be major hurdles in the courtroom; however more effort could be made to make the material accessible. With evaluation and validation of forensic entomology datasets in its relative infancy, defence lawyers, in Texas at least, appear more focused on details such as the quality of temperature data used, blow fly behaviour and timing of developmental events (Michelle Sanford, *personal communication*). Moreover, the most pertinent question from lawyers and jurors seems to simply be "do these data accurately reflect the development of the flies found in this case?". Worryingly, forensic entomologists appear to be less capable of answering this question than desired. Hopefully the current study will motivate future evaluations and validations for other forensically-important species. Likewise, additional developmental datasets are needed; however methods need to be improved with regards to experiment design as a means to provide greater insight as to the accuracy of their applications in criminal investigations. Frameworks and standard operating procedures are an excellent device for promoting system-wide changes and would be a good place to start the process. Such frameworks might incorporate the guidelines laid out in Richards and Villet [4] which focused on the importance of both statistical and biological precision when developing thermal summation (e.g. ADH) models for forensic casework. Furthermore there should be guidance around proper internal validation of the model, with appropriate statistical analysis and a push for the models to be subsequently subject to field evaluation, as demonstrated in the current study. With incorporation of these aspects, entomological evidence be more readily accessible to legal personnel and laypeople.

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APPENDIX A

FIRST APPENDIX - STAGE PREDICTIONS

A.1 Boatright and Tomberlin predictions

Table A.1: Boatright and Tomberlin TOP date minimum estimation and percentage (%) ADH coverage by stage in 2013

BodyID	Month	Milestone	Actual TOP	Pork 20.8° C	Pork24.3° C	Pork 28.2° C	Equine 20.8° C	Equine 24.3° C	Equine 28.2° C
D51	September	1400	22-Sep	20-Sep (92.25)	20-Sep (84.42)	19-Sep (110.78)	20-Sep (98.4)	20-Sep (89.01)	20-Sep (93.24)
D51	September	2000	22-Sep	25-Sep (58.19)	25-Sep (57.91)	24-Sep (75.98)	25-Sep (67.5)	25-Sep (61.05)	25-Sep (63.96)
D51	September	2400	22-Sep	27-Sep (47.12)	27-Sep (46.89)	26-Sep (61.53)	26-Sep (54.66)	27-Sep (49.44)	26-Sep (51.79)
D53	October	1400	10-Oct	11-Oct (76.03)	11-Oct (75.67)	10-Oct (99.29)	11-Oct (88.2)	11-Oct (79.78)	11-Oct (83.58)
D53	October	2000	10-Oct	12-Oct (56.35)	13-Oct (56.08)	12-Oct (73.59)	12-Oct (65.37)	12-Oct (59.13)	12-Oct (61.95)
D53	October	2400	10-Oct	13-Oct (48.12)	13-Oct (47.89)	12-Oct (62.85)	12-Oct (55.83)	13-Oct (50.5)	13-Oct (52.9)
D53	October	3000	10-Oct	18-Oct (36.52)	18-Oct (36.35)	15-Oct (47.7)	17-Oct (42.37)	18-Oct (38.32)	18-Oct (40.15)
D54	October	2000	11-Oct	13-Oct (57.43)	13-Oct (57.16)	12-Oct (75)	13-Oct (66.62)	13-Oct (60.26)	13-Oct (63.13)
D54	October	2400	11-Oct	15-Oct (46.83)	15-Oct (46.61)	14-Oct (61.15)	14-Oct (54.32)	15-Oct (49.14)	15-Oct (51.48)
D54	October	3000	11-Oct	21-Oct (36.35)	21-Oct (36.17)	18-Oct (47.47)	19-Oct (42.16)	20-Oct (38.14)	20-Oct (39.95)

Table A.2: Boatright and Tomberlin TOP date minimum estimation and percentage (%) ADH coverage by stage in 2016

BodyID	Month	Milestone	Actual TOP	Pork 20.8° C	Pork24.3° C	Pork 28.2° C	Equine 20.8° C	Equine 24.3° C	Equine 28.2° C
D06	March	2000	19-Feb	26-Feb (55.13)	27-Feb (54.86)	22-Feb (71.99)	24-Feb (63.95)	25-Feb (57.84)	25-Feb (60.59)
D06	March	2400	19-Feb	29-Feb (46.51)	29-Feb (46.29)	26-Feb (60.74)	28-Feb (53.96)	29-Feb (48.81)	28-Feb (51.13)
D08	March	1400	29-Feb	29-Feb (80.07)	29-Feb (79.69)	26-Feb (104.56)	28-Feb (92.88)	28-Feb (84.01)	28-Feb (88.01)
D08	March	2000	29-Feb	02-Mar (58.52)	02-Mar (58.24)	29-Feb (76.42)	01-Mar (67.88)	02-Mar (61.4)	02-Mar (64.32)
D08	March	2400	29-Feb	06-Mar (46.97)	06-Mar (46.74)	03-Mar (61.33)	05-Mar (54.88)	05-Mar (49.28)	05-Mar (51.63)
D08	March	3000	29-Feb	09-Mar (36.23)	09-Mar (36.06)	06-Mar (47.32)	07-Mar (42.03)	08-Mar (38.02)	08-Mar (39.83)
D23	June	1400	16-Jun	17-Jun (80.6)	17-Jun (80.21)	16-Jun (105.26)	16-Jun (93.5)	17-Jun (84.57)	17-Jun (88.6)
D23	June	2000	16-Jun	18-Jun (59.09)	18-Jun (58.81)	17-Jun (77.17)	18-Jun (68.55)	18-Jun (62.01)	18-Jun (64.96)
D23	June	2400	16-Jun	19-Jun (49.65)	19-Jun (49.42)	18-Jun (64.84)	18-Jun (57.6)	19-Jun (52.1)	18-Jun (54.58)
D23	June	3000	16-Jun	21-Jun (35.87)	21-Jun (35.7)	20-Jun (46.85)	20-Jun (41.61)	20-Jun (37.64)	20-Jun (39.43)
D26	June	1400	21-Jun	22-Jun (85.46)	22-Jun (85.05)	21-Jun (111.6)	21-Jun (99.13)	22-Jun (89.67)	22-Jun (93.93)
D26	June	2000	21-Jun	23-Jun (54.71)	23-Jun (54.45)	23-Jun (71.45)	23-Jun (63.47)	23-Jun (57.41)	23-Jun (60.14)
D26	June	2400	21-Jun	24-Jun (45.3)	24-Jun (45.09)	24-Jun (59.16)	24-Jun (52.55)	24-Jun (47.53)	24-Jun (49.8)
D26	June	3000	21-Jun	26-Jun (38.25)	26-Jun (38.06)	25-Jun (49.95)	25-Jun (44.37)	25-Jun (40.13)	25-Jun (42.04)
D28	June	1400	21-Jun	15-Jun (82.62)	15-Jun (82.22)	14-Jun (107.89)	15-Jun (95.83)	15-Jun (86.69)	15-Jun (90.81)
D30	June	1400	30-Jun	01-Jul (84.78)	01-Jul (84.38)	30-Jun (110.72)	30-Jun (98.35)	01-Jul (88.96)	30-Jun (93.2)
D67	February	1400	26-Jan	31-Jan (76)	31-Jan (75.64)	28-Jan (99.25)	30-Jan (88.16)	31-Jan (79.75)	30-Jan (83.54)
D67	February	2000	26-Jan	05-Feb (54.89)	05-Feb (54.63)	01-Feb (71.68)	02-Feb (63.68)	03-Feb (57.6)	02-Feb (60.34)
D67	February	2400	26-Jan	11-Feb (46.71)	11-Feb (46.48)	06-Feb (60.99)	09-Feb (54.18)	10-Feb (49.01)	10-Feb (51.34)

Table A.3: Boatright and Tomberlin TOP date minimum estimation and percentage (%) ADH coverage by stage in 2015

BodyID	Month	Milestone	Actual TOP	Pork 20.8° C	Pork24.3° C	Pork 28.2° C	Equine 20.8° C	Equine 24.3° C	Equine 28.2° C
D23	June	1400	08-Jun	10-Jun (71.24)	10-Jun (70.9)	09-Jun (93.03)	09-Jun (82.64)	09-Jun (74.75)	09-Jun (78.31)
D23	June	2000	08-Jun	11-Jun (55.12)	11-Jun (54.86)	10-Jun (63.94)	10-Jun (63.94)	11-Jun (57.84)	10-Jun (60.59)
D23	June	2400	08-Jun	12-Jun (46)	12-Jun (45.78)	11-Jun (60.08)	11-Jun (53.36)	11-Jun (48.27)	11-Jun (50.57)
D23	June	3000	08-Jun	13-Jun (35.51)	13-Jun (35.34)	12-Jun (46.37)	13-Jun (41.19)	13-Jun (37.26)	13-Jun (39.03)
D24	June	1400	08-Jun	09-Jun (71.24)	09-Jun (70.9)	09-Jun (93.03)	09-Jun (82.64)	09-Jun (74.75)	09-Jun (78.31)
D24	June	2000	08-Jun	10-Jun (55.12)	10-Jun (54.86)	10-Jun (63.94)	10-Jun (63.94)	10-Jun (57.84)	10-Jun (60.59)
D24	June	2400	08-Jun	11-Jun (45.75)	11-Jun (45.53)	10-Jun (59.74)	11-Jun (53.07)	11-Jun (48)	11-Jun (50.29)
D24	June	3000	08-Jun	13-Jun (35.51)	13-Jun (35.34)	12-Jun (46.37)	13-Jun (41.19)	13-Jun (37.26)	13-Jun (39.03)
D25	June	1400	08-Jun	09-Jun (71.24)	09-Jun (70.9)	09-Jun (93.03)	09-Jun (82.64)	09-Jun (74.75)	09-Jun (78.31)
D25	June	2000	08-Jun	10-Jun (55.12)	10-Jun (55.7)	10-Jun (63.94)	10-Jun (64.92)	10-Jun (58.73)	10-Jun (61.52)
D25	June	2400	08-Jun	11-Jun (45.75)	11-Jun (45.53)	11-Jun (53.07)	11-Jun (48)	11-Jun (50.29)	11-Jun (52.99)
D27	June	1400	05-Jun	06-Jun (73.26)	06-Jun (72.91)	05-Jun (95.67)	06-Jun (84.98)	06-Jun (76.87)	06-Jun (80.53)
D27	June	2000	05-Jun	07-Jun (55.68)	07-Jun (55.42)	06-Jun (72.71)	07-Jun (64.59)	07-Jun (58.43)	07-Jun (61.21)
D27	June	2400	05-Jun	08-Jun (46.23)	08-Jun (46.01)	08-Jun (60.38)	08-Jun (53.63)	08-Jun (48.51)	08-Jun (50.82)
D27	June	3000	05-Jun	10-Jun (37.37)	10-Jun (37.19)	09-Jun (48.8)	09-Jun (43.35)	10-Jun (39.21)	09-Jun (41.07)
D28	June	1400	04-Jun	05-Jun (74.42)	05-Jun (74.07)	04-Jun (97.19)	05-Jun (86.33)	05-Jun (78.09)	05-Jun (81.81)
D28	June	2000	04-Jun	06-Jun (59.09)	06-Jun (58.81)	05-Jun (77.16)	06-Jun (68.54)	06-Jun (62)	06-Jun (64.95)
D28	June	2400	04-Jun	07-Jun (46.86)	07-Jun (46.64)	07-Jun (61.2)	07-Jun (54.36)	07-Jun (49.17)	07-Jun (51.51)
D30	June	1400	04-Jun	09-Jun (73.27)	09-Jun (72.92)	09-Jun (95.69)	09-Jun (85)	09-Jun (76.88)	09-Jun (80.54)
D30	June	2000	08-Jun	11-Jun (56.02)	11-Jun (55.75)	10-Jun (73.15)	10-Jun (64.98)	11-Jun (58.78)	10-Jun (61.57)
D30	June	m 2400	08-Jun	12-Jun (47.48)	12-Jun (47.25)	11-Jun (62)	11-Jun (55.08)	11-Jun (49.82)	11-Jun (52.19)
D35	July	1400	02-Jul	02-Jul (84.2)	02-Jul (83.8)	01-Jul (109.96)	02-Jul (97.68)	02-Jul (88.35)	02-Jul (92.56)
D35	July	2000	02-Jul	04-Jul (54.92)	04-Jul (54.66)	04-Jul (71.72)	04-Jul (63.71)	04-Jul (57.63)	04-Jul (60.37)
D35	July	2400	02-Jul	06-Jul (45.04)	06-Jul (44.83)	05-Jul (58.82)	05-Jul (52.25)	05-Jul (47.26)	05-Jul (49.51)
D35	July	3000	02-Jul	07-Jul (37.57)	07-Jul (37.39)	06-Jul (49.07)	06-Jul (43.59)	07-Jul (39.43)	07-Jul (41.3)
D39	July	1400	15-Jul	16-Jul (77.15)	16-Jul (76.78)	15-Jul (100.75)	15-Jul (89.5)	16-Jul (80.96)	16-Jul (84.81)
D39	July	2000	15-Jul	17-Jul (57.07)	17-Jul (56.8)	16-Jul (74.53)	17-Jul (66.21)	17-Jul (59.89)	17-Jul (62.74)
D39	July	2400	15-Jul	18-Jul (46.53)	18-Jul (46.31)	17-Jul (60.77)	18-Jul (53.98)	18-Jul (48.83)	18-Jul (51.15)
D40	August	2000	29-Jul	31-Jul (56.91)	31-Jul (56.64)	30-Jul (74.32)	31-Jul (66.01)	31-Jul (59.71)	31-Jul (62.56)
D43	August	1400	12-Aug	13-Aug (74.68)	13-Aug (74.32)	12-Aug (97.52)	13-Aug (86.63)	13-Aug (78.36)	13-Aug (82.09)
D43	August	2000	12-Aug	14-Aug (53.49)	14-Aug (53.24)	13-Aug (69.86)	14-Aug (62.05)	14-Aug (56.13)	14-Aug (58.8)
D43	August	2400	12-Aug	15-Aug (45.96)	15-Aug (45.74)	14-Aug (60.02)	14-Aug (53.32)	15-Aug (48.23)	14-Aug (50.52)
D45	September	1400	28-Aug	29-Aug (74.95)	29-Aug (74.59)	29-Aug (97.87)	29-Aug (86.94)	29-Aug (78.64)	29-Aug (82.38)
D45	September	2000	28-Aug	30-Aug (58.1)	31-Aug (57.82)	30-Aug (75.87)	30-Aug (67.39)	30-Aug (60.96)	30-Aug (63.86)
D45	September	2400	28-Aug	01-Sep (46.98)	01-Sep (46.76)	31-Aug (61.35)	31-Aug (54.5)	31-Aug (49.3)	31-Aug (51.64)
D51	September	2000	18-Sep	20-Sep (55.86)	20-Sep (55.6)	19-Sep (72.95)	20-Sep (64.8)	20-Sep (58.62)	20-Sep (61.41)
D51	September	2400	18-Sep	21-Sep (46.63)	21-Sep (46.41)	20-Sep (60.9)	21-Sep (54.09)	21-Sep (48.93)	21-Sep (51.26)
D53	October	1400	02-Oct	03-Oct (74.89)	03-Oct (74.53)	02-Oct (97.8)	03-Oct (86.87)	03-Oct (78.58)	03-Oct (82.32)
D53	October	2400	02-Oct	07-Oct (43.01)	07-Oct (42.8)	06-Oct (56.16)	06-Oct (49.89)	07-Oct (45.12)	07-Oct (47.27)
D54	October	1400	02-Oct	03-Oct (76.59)	03-Oct (76.22)	02-Oct (100.02)	03-Oct (88.84)	03-Oct (80.36)	03-Oct (84.19)
D54	October	2000	02-Oct	05-Oct (58.52)	05-Oct (58.24)	04-Oct (76.42)	04-Oct (67.88)	05-Oct (61.4)	05-Oct (64.33)
D54	October	2400	02-Oct	06-Oct (51.22)	06-Oct (50.98)	05-Oct (66.89)	05-Oct (59.42)	06-Oct (53.74)	05-Oct (56.3)
D54	October	3000	02-Oct	08-Oct (37.99)	08-Oct (37.81)	07-Oct (49.61)	08-Oct (44.07)	08-Oct (39.86)	08-Oct (41.76)
D61	November	1400	06-Nov	08-Nov (78.34)	08-Nov (77.96)	06-Nov (102.3)	07-Nov (90.87)	08-Nov (82.2)	07-Nov (86.11)
D61	November	2000	06-Nov	11-Nov (56)	11-Nov (55.74)	10-Nov (73.13)	11-Nov (64.96)	11-Nov (58.76)	11-Nov (61.56)
D61	November	2400	06-Nov	13-Nov (47.09)	13-Nov (46.86)	11-Nov (61.49)	12-Nov (54.62)	13-Nov (49.41)	12-Nov (51.76)
D61	November	3000	06-Nov	17-Nov (36.66)	17-Nov (36.48)	16-Nov (47.87)	16-Nov (42.52)	17-Nov (38.46)	17-Nov (40.3)

Table A.4: Boatright and Tomberlin TOP date mean estimation and percentage (%) ADH coverage by stage in 2013

BodyID	Month	Milestone	Actual TOP	Pork 20.8°C	Pork24.3°C	Pork 28.2°C	Equine 20.8°C	Equine 24.3°C	Equine 28.2°C
D51	September	1400	22-Sep	20-Sep (92.25)	20-Sep (91.7)	19-Sep (120.72)	20-Sep (110.59)	20-Sep (100.83)	20-Sep (122.16)
D51	September	2000	22-Sep	25-Sep (63.28)	25-Sep (62.9)	24-Sep (82.81)	25-Sep (75.86)	25-Sep (69.16)	25-Sep (83.79)
D51	September	2400	22-Sep	27-Sep (51.24)	27-Sep (50.93)	26-Sep (67.06)	26-Sep (61.43)	26-Sep (56.01)	26-Sep (67.86)
D53	October	1400	10-Oct	11-Oct (82.69)	11-Oct (82.19)	10-Oct (108.21)	10-Oct (99.13)	11-Oct (90.38)	10-Oct (109.5)
D53	October	2000	10-Oct	12-Oct (61.28)	12-Oct (60.92)	11-Oct (80.2)	12-Oct (73.47)	12-Oct (66.99)	12-Oct (81.15)
D53	October	2400	10-Oct	13-Oct (52.34)	13-Oct (52.02)	12-Oct (68.49)	12-Oct (62.74)	13-Oct (57.2)	12-Oct (69.3)
D53	October	3000	10-Oct	18-Oct (39.72)	18-Oct (39.48)	15-Oct (51.98)	16-Oct (47.62)	17-Oct (43.42)	16-Oct (52.6)
D54	October	2000	11-Oct	13-Oct (62.46)	13-Oct (62.08)	12-Oct (81.74)	12-Oct (74.88)	13-Oct (68.27)	12-Oct (82.71)
D54	October	2400	11-Oct	15-Oct (50.93)	15-Oct (50.62)	14-Oct (66.64)	14-Oct (61.05)	15-Oct (55.66)	14-Oct (67.44)
D54	October	3000	11-Oct	20-Oct (39.53)	20-Oct (39.29)	17-Oct (51.73)	18-Oct (47.39)	20-Oct (43.21)	18-Oct (52.34)

Table A.5: Boatright and Tomberlin TOP date mean estimation and percentage (%) ADH coverage by stage in 2016

BodyID	Month	Milestone	Actual TOP	Pork 20.8°C	Pork24.3°C	Pork 28.2°C	Equine 20.8°C	Equine 24.3°C	Equine 28.2°C
D06	March	2000	19-Feb	25-Feb (59.95)	25-Feb (59.59)	22-Feb (78.45)	23-Feb (71.87)	25-Feb (65.53)	23-Feb (79.38)
D06	March	2400	19-Feb	28-Feb (50.58)	28-Feb (50.28)	25-Feb (66.2)	27-Feb (60.64)	28-Feb (55.29)	27-Feb (66.98)
D08	March	1400	29-Feb	28-Feb (87.07)	28-Feb (86.55)	26-Feb (113.95)	27-Feb (104.39)	28-Feb (95.17)	27-Feb (115.3)
D08	March	2000	29-Feb	02-Mar (63.64)	02-Mar (63.26)	28-Feb (83.28)	29-Feb (76.29)	01-Mar (69.56)	29-Feb (84.27)
D08	March	2400	29-Feb	05-Mar (51.08)	05-Mar (50.77)	03-Mar (66.84)	04-Mar (61.23)	05-Mar (55.83)	04-Mar (67.64)
D08	March	3000	29-Feb	08-Mar (39.41)	08-Mar (39.17)	06-Mar (51.57)	07-Mar (47.24)	08-Mar (43.07)	07-Mar (52.18)
D23	June	1400	16-Jun	17-Jun (87.65)	17-Jun (87.13)	16-Jun (114.7)	16-Jun (105.08)	17-Jun (95.81)	16-Jun (116.07)
D23	June	2000	16-Jun	18-Jun (64.26)	18-Jun (63.88)	17-Jun (84.1)	17-Jun (77.04)	18-Jun (70.24)	17-Jun (85.1)
D23	June	2400	16-Jun	18-Jun (54)	18-Jun (53.67)	18-Jun (70.66)	18-Jun (64.73)	18-Jun (59.02)	18-Jun (71.5)
D23	June	3000	16-Jun	20-Jun (39.01)	20-Jun (38.78)	20-Jun (51.05)	20-Jun (46.77)	20-Jun (42.64)	20-Jun (51.66)
D26	June	1400	21-Jun	22-Jun (92.93)	22-Jun (92.38)	21-Jun (121.62)	21-Jun (111.41)	22-Jun (101.58)	21-Jun (123.06)
D26	June	2000	21-Jun	23-Jun (59.5)	23-Jun (59.15)	23-Jun (77.87)	23-Jun (71.33)	23-Jun (65.04)	23-Jun (78.79)
D26	June	2400	21-Jun	24-Jun (49.27)	24-Jun (48.97)	23-Jun (64.47)	24-Jun (59.06)	24-Jun (53.85)	24-Jun (65.24)
D26	June	3000	21-Jun	25-Jun (41.59)	25-Jun (41.34)	25-Jun (54.43)	25-Jun (49.86)	25-Jun (45.46)	25-Jun (55.08)
D28	June	1400	21-Jun	15-Jun (89.84)	15-Jun (89.31)	14-Jun (117.57)	14-Jun (107.71)	15-Jun (98.2)	14-Jun (118.97)
D30	June	1400	30-Jun	01-Jul (92.2)	01-Jul (91.65)	30-Jun (120.66)	30-Jun (110.54)	30-Jun (100.78)	30-Jun (122.1)
D67	February	1400	26-Jan	30-Jan (82.65)	31-Jan (82.16)	27-Jan (108.16)	29-Jan (99.09)	30-Jan (90.34)	29-Jan (109.45)
D67	February	2000	26-Jan	02-Feb (59.7)	02-Feb (59.34)	31-Jan (78.12)	01-Feb (71.57)	02-Feb (65.25)	01-Feb (79.05)
D67	February	2400	26-Jan	10-Feb (50.79)	10-Feb (50.49)	05-Feb (66.47)	07-Feb (60.89)	10-Feb (55.52)	08-Feb (67.26)

Table A.6: Boatright and Tomberlin TOP date mean estimation and percentage (%) ADH coverage by stage in 2015

BodyID	Month	Milestone	Actual TOP	Pork 20.8 °C	Pork 24.3 °C	Pork 28.2 °C	Equine 20.8 °C	Equine 24.3 °C	Equine 28.2 °C
D23	June	1400	08-Jun	09-Jun (77.47)	09-Jun (77.01)	09-Jun (101.39)	09-Jun (92.88)	09-Jun (84.68)	09-Jun (102.59)
D23	June	2000	08-Jun	10-Jun (59.95)	10-Jun (59.59)	10-Jun (71.87)	10-Jun (71.87)	10-Jun (65.52)	10-Jun (79.38)
D23	June	2400	08-Jun	11-Jun (50.03)	11-Jun (49.73)	11-Jun (59.98)	11-Jun (59.98)	11-Jun (54.68)	11-Jun (66.25)
D23	June	3000	08-Jun	13-Jun (38.62)	13-Jun (38.39)	13-Jun (46.3)	13-Jun (46.3)	13-Jun (42.21)	13-Jun (51.14)
D24	June	1400	08-Jun	09-Jun (77.47)	09-Jun (77.01)	08-Jun (101.38)	09-Jun (92.88)	09-Jun (84.68)	09-Jun (102.59)
D24	June	2000	08-Jun	10-Jun (59.95)	10-Jun (59.59)	09-Jun (78.45)	10-Jun (78.45)	10-Jun (65.52)	10-Jun (79.38)
D24	June	2400	08-Jun	11-Jun (49.75)	11-Jun (49.45)	10-Jun (65.11)	11-Jun (59.64)	11-Jun (54.38)	11-Jun (65.88)
D24	June	3000	08-Jun	13-Jun (38.62)	13-Jun (38.39)	12-Jun (46.3)	12-Jun (46.3)	13-Jun (42.21)	12-Jun (51.14)
D25	June	1400	08-Jun	09-Jun (77.47)	09-Jun (77.01)	08-Jun (101.38)	09-Jun (92.88)	09-Jun (84.68)	09-Jun (102.59)
D25	June	2000	08-Jun	10-Jun (60.87)	10-Jun (60.5)	09-Jun (79.65)	10-Jun (72.97)	10-Jun (66.53)	10-Jun (80.6)
D25	June	2400	08-Jun	11-Jun (49.75)	11-Jun (49.45)	10-Jun (65.11)	11-Jun (59.64)	11-Jun (54.38)	11-Jun (65.88)
D27	June	1400	05-Jun	06-Jun (79.67)	06-Jun (79.19)	05-Jun (104.26)	05-Jun (95.51)	06-Jun (87.08)	05-Jun (105.5)
D27	June	2000	05-Jun	07-Jun (60.55)	07-Jun (60.19)	06-Jun (79.24)	07-Jun (72.59)	07-Jun (66.19)	07-Jun (80.19)
D27	June	2400	05-Jun	08-Jun (50.28)	08-Jun (49.98)	07-Jun (65.8)	08-Jun (60.28)	08-Jun (54.96)	08-Jun (66.58)
D28	June	1400	04-Jun	05-Jun (80.94)	05-Jun (80.45)	04-Jun (105.92)	04-Jun (97.03)	05-Jun (88.47)	04-Jun (107.18)
D28	June	2000	04-Jun	06-Jun (64.26)	06-Jun (63.87)	05-Jun (84.09)	05-Jun (77.03)	06-Jun (70.24)	05-Jun (85.09)
D28	June	2400	04-Jun	07-Jun (50.96)	07-Jun (50.66)	06-Jun (66.69)	07-Jun (61.1)	07-Jun (55.7)	07-Jun (67.49)
D30	June	1400	08-Jun	09-Jun (79.68)	09-Jun (79.21)	09-Jun (104.28)	09-Jun (95.53)	09-Jun (87.1)	09-Jun (105.52)
D30	June	2000	08-Jun	10-Jun (60.92)	10-Jun (60.55)	10-Jun (79.72)	10-Jun (73.03)	10-Jun (66.58)	10-Jun (80.67)
D30	June	2400	08-Jun	11-Jun (51.63)	11-Jun (51.33)	11-Jun (67.57)	11-Jun (61.9)	11-Jun (56.44)	11-Jun (68.38)
D35	July	1400	02-Jul	02-Jul (91.57)	02-Jul (91.02)	01-Jul (119.83)	02-Jul (109.78)	02-Jul (100.09)	02-Jul (121.26)
D35	July	2000	02-Jul	04-Jul (59.72)	04-Jul (59.37)	03-Jul (78.16)	04-Jul (71.6)	04-Jul (65.28)	04-Jul (79.09)
D35	July	2400	02-Jul	05-Jul (48.98)	05-Jul (48.69)	05-Jul (64.1)	05-Jul (58.72)	05-Jul (53.54)	05-Jul (64.86)
D35	July	3000	02-Jul	07-Jul (40.86)	07-Jul (40.62)	06-Jul (53.47)	06-Jul (48.99)	06-Jul (44.66)	06-Jul (54.11)
D39	July	1400	15-Jul	16-Jul (83.9)	16-Jul (83.4)	15-Jul (109.8)	15-Jul (100.59)	16-Jul (91.71)	15-Jul (111.11)
D39	July	2000	15-Jul	17-Jul (62.07)	17-Jul (61.7)	16-Jul (81.22)	16-Jul (74.41)	17-Jul (67.84)	16-Jul (82.19)
D39	July	2400	15-Jul	18-Jul (50.61)	18-Jul (50.3)	17-Jul (66.23)	17-Jul (60.67)	18-Jul (55.31)	17-Jul (67.01)
D40	August	2000	29-Jul	31-Jul (61.89)	31-Jul (61.52)	30-Jul (80.99)	31-Jul (74.19)	31-Jul (67.65)	31-Jul (81.95)
D43	August	1400	12-Aug	13-Aug (81.21)	13-Aug (80.72)	12-Aug (106.28)	12-Aug (97.36)	13-Aug (88.77)	12-Aug (107.54)
D43	August	2000	12-Aug	14-Aug (58.17)	14-Aug (57.83)	13-Aug (76.13)	13-Aug (69.74)	14-Aug (63.59)	13-Aug (77.04)
D43	August	2400	12-Aug	15-Aug (49.99)	15-Aug (49.69)	14-Aug (65.41)	14-Aug (59.93)	14-Aug (54.64)	14-Aug (66.19)
D45	September	1400	28-Aug	29-Aug (81.5)	29-Aug (81.02)	29-Aug (106.66)	29-Aug (97.71)	29-Aug (89.09)	29-Aug (107.93)
D45	September	2000	28-Aug	30-Aug (63.18)	30-Aug (62.8)	30-Aug (82.68)	30-Aug (75.74)	30-Aug (69.06)	30-Aug (83.66)
D45	September	2400	28-Aug	31-Aug (51.09)	31-Aug (50.79)	31-Aug (66.86)	31-Aug (61.25)	31-Aug (55.85)	31-Aug (67.66)
D51	September	2000	18-Sep	20-Sep (60.75)	20-Sep (60.39)	19-Sep (79.5)	20-Sep (72.83)	20-Sep (66.4)	20-Sep (80.45)
D51	September	2400	18-Sep	21-Sep (50.71)	21-Sep (50.41)	20-Sep (66.36)	21-Sep (60.79)	21-Sep (55.43)	21-Sep (67.15)
D53	October	1400	02-Oct	03-Oct (81.44)	03-Oct (80.95)	02-Oct (106.58)	02-Oct (97.64)	03-Oct (89.02)	02-Oct (107.85)
D53	October	2000	02-Oct	07-Oct (46.77)	07-Oct (46.49)	06-Oct (61.2)	06-Oct (56.07)	06-Oct (51.12)	06-Oct (61.93)
D54	October	1400	02-Oct	03-Oct (83.29)	03-Oct (82.79)	02-Oct (109)	02-Oct (99.85)	03-Oct (91.04)	02-Oct (110.29)
D54	October	2000	02-Oct	05-Oct (63.64)	05-Oct (63.26)	04-Oct (83.28)	04-Oct (76.29)	05-Oct (69.56)	04-Oct (84.27)
D54	October	2400	02-Oct	05-Oct (55.7)	05-Oct (55.37)	04-Oct (72.89)	05-Oct (66.78)	05-Oct (60.88)	05-Oct (73.76)
D54	October	3000	02-Oct	08-Oct (41.31)	08-Oct (41.06)	07-Oct (54.06)	07-Oct (49.52)	08-Oct (45.15)	07-Oct (54.7)

Table A.7: Boatright and Tomberlin TOP date maximum estimation and percentage (%) ADH coverage by stage in 2013

BodyID	Month	Milestone	Actual TOP	Pork 20.8° C	Pork24.3° C	Pork 28.2° C	Equine 20.8° C	Equine 24.3° C	Equine 28.2° C
D51	September	1400	22-Sep	20-Sep (98.01)	20-Sep (98.97)	19-Sep (120.72)	19-Sep (110.59)	20-Sep (100.83)	19-Sep (122.16)
D51	September	2000	22-Sep	25-Sep (67.23)	25-Sep (67.89)	24-Sep (82.81)	24-Sep (75.86)	25-Sep (69.16)	24-Sep (83.79)
D51	September	2400	22-Sep	26-Sep (54.44)	26-Sep (54.98)	25-Sep (67.06)	26-Sep (61.43)	26-Sep (56.01)	25-Sep (67.86)
D53	October	1400	10-Oct	11-Oct (87.85)	11-Oct (88.71)	10-Oct (108.21)	10-Oct (99.13)	11-Oct (90.38)	10-Oct (109.5)
D53	October	2000	10-Oct	12-Oct (65.11)	12-Oct (65.75)	11-Oct (80.2)	12-Oct (73.47)	12-Oct (66.99)	11-Oct (81.15)
D53	October	2400	10-Oct	12-Oct (55.61)	12-Oct (56.15)	12-Oct (68.49)	12-Oct (62.74)	12-Oct (57.2)	12-Oct (69.3)
D53	October	3000	10-Oct	17-Oct (42.2)	17-Oct (42.61)	15-Oct (51.98)	15-Oct (47.62)	17-Oct (43.42)	15-Oct (52.6)
D54	October	2000	11-Oct	13-Oct (66.36)	13-Oct (67.01)	12-Oct (81.74)	12-Oct (74.88)	13-Oct (68.27)	12-Oct (82.71)
D54	October	2400	11-Oct	14-Oct (54.11)	14-Oct (54.64)	13-Oct (66.64)	14-Oct (61.05)	14-Oct (55.66)	13-Oct (67.44)
D54	October	3000	11-Oct	19-Oct (42)	19-Oct (42.41)	17-Oct (51.73)	18-Oct (47.39)	19-Oct (43.21)	17-Oct (52.34)

Table A.8: Boatright and Tomberlin TOP date maximum estimation and percentage (%) ADH coverage by stage in 2016

BodyID	Month	Milestone	Actual TOP	Pork 20.8° C	Pork24.3° C	Pork 28.2° C	Equine 20.8° C	Equine 24.3° C	Equine 28.2° C
D06	March	2000	19-Feb	24-Feb (63.69)	23-Feb (64.32)	22-Feb (78.45)	22-Feb (71.87)	23-Feb (65.53)	21-Feb (79.38)
D06	March	2400	19-Feb	28-Feb (53.74)	28-Feb (54.27)	24-Feb (66.2)	26-Feb (60.64)	28-Feb (55.29)	24-Feb (66.98)
D08	March	1400	29-Feb	28-Feb (92.51)	27-Feb (93.42)	25-Feb (113.95)	26-Feb (104.39)	27-Feb (95.17)	25-Feb (115.3)
D08	March	2000	29-Feb	01-Mar (67.61)	01-Mar (68.27)	28-Feb (83.28)	29-Feb (76.29)	01-Mar (69.56)	28-Feb (84.27)
D08	March	2400	29-Feb	05-Mar (54.27)	04-Mar (54.8)	03-Mar (66.84)	04-Mar (61.23)	04-Mar (55.83)	02-Mar (67.64)
D08	March	3000	29-Feb	08-Mar (41.87)	07-Mar (42.28)	06-Mar (51.57)	06-Mar (47.24)	07-Mar (43.07)	05-Mar (52.18)
D23	June	1400	16-Jun	16-Jun (93.13)	16-Jun (94.04)	16-Jun (114.7)	16-Jun (105.08)	16-Jun (95.81)	16-Jun (116.07)
D23	June	2000	16-Jun	18-Jun (68.28)	18-Jun (68.95)	17-Jun (84.1)	17-Jun (77.04)	18-Jun (70.24)	17-Jun (85.1)
D23	June	2400	16-Jun	18-Jun (57.37)	18-Jun (57.93)	18-Jun (70.66)	18-Jun (64.73)	18-Jun (59.02)	18-Jun (71.5)
D23	June	3000	16-Jun	20-Jun (41.45)	20-Jun (41.85)	20-Jun (51.05)	20-Jun (46.77)	20-Jun (42.64)	19-Jun (51.66)
D26	June	1400	21-Jun	21-Jun (98.74)	21-Jun (99.7)	21-Jun (121.62)	21-Jun (111.41)	21-Jun (101.58)	21-Jun (123.06)
D26	June	2000	21-Jun	23-Jun (63.22)	23-Jun (63.84)	22-Jun (77.87)	23-Jun (71.33)	23-Jun (65.04)	22-Jun (78.79)
D26	June	2400	21-Jun	24-Jun (52.34)	24-Jun (52.85)	23-Jun (64.47)	24-Jun (59.06)	24-Jun (53.85)	23-Jun (65.24)
D26	June	3000	21-Jun	25-Jun (44.19)	24-Jun (44.62)	24-Jun (54.43)	25-Jun (49.86)	25-Jun (45.46)	24-Jun (55.08)
D28	June	1400	21-Jun	15-Jun (95.46)	15-Jun (96.39)	14-Jun (117.57)	14-Jun (107.71)	15-Jun (98.2)	14-Jun (118.97)
D30	June	1400	30-Jun	30-Jun (97.96)	30-Jun (98.92)	30-Jun (120.66)	30-Jun (110.54)	30-Jun (100.78)	30-Jun (122.1)
D67	February	1400	26-Jan	30-Jan (87.82)	30-Jan (88.67)	25-Jan (108.16)	28-Jan (99.09)	30-Jan (90.34)	25-Jan (109.45)
D67	February	2000	26-Jan	02-Feb (63.43)	02-Feb (64.05)	31-Jan (78.12)	01-Feb (71.57)	02-Feb (65.25)	31-Jan (79.05)
D67	February	2400	26-Jan	09-Feb (53.97)	09-Feb (54.49)	02-Feb (66.47)	06-Feb (60.89)	09-Feb (55.52)	02-Feb (67.26)

Table A.9: Boatright and Tomberlin TOP date maximum estimation and percentage (%) ADH coverage by stage in 2015

BodyID	Month	Milestone	Actual TOP	Pork 20.8 °C	Pork 24.3 °C	Pork 28.2 °C	Equine 20.8 °C	Equine 24.3 °C	Equine 28.2 °C
D23	June	1400	8-Jun	09-Jun (82.31)	09-Jun (83.12)	08-Jun (101.39)	09-Jun (92.88)	09-Jun (84.68)	08-Jun (102.59)
D23	June	2000	8-Jun	10-Jun (63.69)	10-Jun (64.32)	10-Jun (78.45)	10-Jun (71.87)	10-Jun (79.38)	10-Jun (65.52)
D23	June	2400	8-Jun	11-Jun (53.15)	11-Jun (53.67)	10-Jun (65.47)	11-Jun (59.98)	11-Jun (54.68)	11-Jun (66.25)
D23	June	3000	8-Jun	13-Jun (41.03)	13-Jun (41.43)	12-Jun (50.54)	12-Jun (46.3)	13-Jun (42.21)	12-Jun (51.14)
D24	June	1400	8-Jun	09-Jun (82.31)	09-Jun (83.12)	08-Jun (101.38)	09-Jun (92.88)	09-Jun (84.68)	08-Jun (102.59)
D24	June	2000	8-Jun	10-Jun (63.69)	10-Jun (64.32)	09-Jun (78.45)	10-Jun (71.87)	10-Jun (65.52)	09-Jun (79.38)
D24	June	2400	8-Jun	11-Jun (52.86)	11-Jun (53.38)	10-Jun (65.11)	10-Jun (59.64)	11-Jun (54.38)	10-Jun (65.88)
D24	June	3000	8-Jun	13-Jun (41.03)	13-Jun (41.43)	12-Jun (50.54)	12-Jun (46.3)	13-Jun (42.21)	12-Jun (51.14)
D25	June	1400	09-Jun	09-Jun (82.31)	09-Jun (83.12)	08-Jun (101.38)	09-Jun (92.88)	09-Jun (84.68)	08-Jun (102.59)
D25	June	2000	8-Jun	10-Jun (64.67)	10-Jun (65.3)	09-Jun (79.65)	10-Jun (72.97)	10-Jun (66.53)	09-Jun (80.6)
D25	June	2400	8-Jun	11-Jun (52.86)	11-Jun (53.38)	10-Jun (65.11)	11-Jun (59.64)	11-Jun (54.38)	10-Jun (65.88)
D27	June	1400	06-Jun	06-Jun (84.65)	06-Jun (85.47)	05-Jun (104.26)	05-Jun (95.51)	06-Jun (87.08)	05-Jun (105.5)
D27	June	2000	07-Jun	07-Jun (64.34)	07-Jun (64.97)	06-Jun (79.24)	06-Jun (72.59)	07-Jun (66.19)	06-Jun (80.19)
D27	June	2400	08-Jun	08-Jun (53.42)	08-Jun (53.94)	07-Jun (65.8)	08-Jun (60.28)	08-Jun (54.96)	07-Jun (66.58)
D27	June	3000	09-Jun	09-Jun (43.18)	09-Jun (43.6)	09-Jun (53.18)	09-Jun (48.72)	09-Jun (44.42)	09-Jun (53.81)
D28	June	1400	05-Jun	05-Jun (85.99)	05-Jun (86.83)	04-Jun (105.92)	04-Jun (97.03)	04-Jun (88.47)	04-Jun (107.18)
D28	June	2000	06-Jun	06-Jun (68.27)	06-Jun (68.94)	05-Jun (84.09)	05-Jun (77.03)	06-Jun (70.24)	05-Jun (85.09)
D28	June	2400	07-Jun	07-Jun (54.15)	07-Jun (54.68)	06-Jun (66.69)	07-Jun (61.1)	07-Jun (55.7)	06-Jun (67.49)
D30	June	1400	09-Jun	09-Jun (84.66)	09-Jun (85.49)	08-Jun (104.28)	09-Jun (95.53)	09-Jun (87.1)	08-Jun (105.52)
D30	June	2000	10-Jun	10-Jun (64.72)	10-Jun (65.36)	10-Jun (79.72)	10-Jun (73.03)	10-Jun (66.58)	10-Jun (80.67)
D30	June	2400	11-Jun	11-Jun (54.86)	11-Jun (55.4)	10-Jun (67.57)	11-Jun (61.9)	11-Jun (56.44)	10-Jun (68.38)
D35	July	1400	02-Jul	02-Jul (97.29)	02-Jul (98.24)	01-Jul (119.83)	01-Jul (109.78)	02-Jul (100.09)	01-Jul (121.26)
D35	July	2000	2-Jul	04-Jul (63.46)	04-Jul (64.08)	03-Jul (78.16)	04-Jul (71.6)	04-Jul (65.28)	03-Jul (79.09)
D35	July	2400	2-Jul	05-Jul (52.04)	05-Jul (52.55)	04-Jul (64.1)	05-Jul (58.72)	05-Jul (53.54)	04-Jul (64.86)
D35	July	3000	2-Jul	06-Jul (43.41)	06-Jul (43.84)	06-Jul (53.47)	06-Jul (48.99)	06-Jul (44.66)	06-Jul (54.11)
D39	July	1400	15-Jul	15-Jul (89.15)	15-Jul (90.02)	15-Jul (109.8)	15-Jul (100.59)	15-Jul (91.71)	15-Jul (111.11)
D39	July	2000	15-Jul	17-Jul (65.95)	17-Jul (66.59)	16-Jul (81.22)	16-Jul (74.41)	17-Jul (67.84)	16-Jul (82.19)
D39	July	2400	15-Jul	18-Jul (53.77)	18-Jul (54.29)	17-Jul (66.23)	17-Jul (60.67)	17-Jul (55.31)	17-Jul (67.01)
D40	August	2000	29-Jul	31-Jul (65.76)	31-Jul (66.4)	30-Jul (80.99)	30-Jul (74.19)	31-Jul (67.65)	30-Jul (81.95)
D43	August	1400	12-Aug	13-Aug (86.29)	13-Aug (87.13)	12-Aug (106.28)	12-Aug (97.36)	13-Aug (88.77)	12-Aug (107.54)
D43	August	2000	12-Aug	14-Aug (61.81)	14-Aug (62.41)	13-Aug (76.13)	13-Aug (69.74)	14-Aug (63.59)	13-Aug (77.04)
D43	August	2400	12-Aug	14-Aug (53.11)	14-Aug (53.63)	14-Aug (65.41)	14-Aug (59.93)	14-Aug (54.64)	14-Aug (66.19)
D45	September	1400	28-Aug	29-Aug (86.6)	29-Aug (87.44)	28-Aug (106.66)	29-Aug (97.71)	29-Aug (89.09)	28-Aug (107.93)
D45	September	2000	28-Aug	30-Aug (67.13)	30-Aug (67.78)	29-Aug (82.68)	30-Aug (75.74)	30-Aug (69.06)	29-Aug (83.66)
D45	September	2400	28-Aug	31-Aug (54.28)	31-Aug (54.81)	30-Aug (66.86)	31-Aug (61.25)	31-Aug (55.85)	30-Aug (67.66)
D51	September	2000	18-Sep	20-Sep (64.55)	20-Sep (65.18)	19-Sep (79.5)	19-Sep (72.83)	20-Sep (66.4)	19-Sep (80.45)
D51	September	2400	18-Sep	21-Sep (53.88)	21-Sep (54.41)	20-Sep (66.36)	20-Sep (60.79)	21-Sep (55.43)	20-Sep (67.15)
D53	October	1400	2-Oct	03-Oct (86.55)	03-Oct (87.38)	02-Oct (106.58)	02-Oct (97.64)	03-Oct (89.02)	02-Oct (107.85)
D53	October	2000	2-Oct	06-Oct (49.69)	06-Oct (50.18)	05-Oct (61.2)	06-Oct (56.07)	06-Oct (51.12)	05-Oct (61.93)
D54	October	1400	2-Oct	03-Oct (88.49)	03-Oct (89.36)	02-Oct (109)	02-Oct (99.85)	03-Oct (91.04)	02-Oct (110.29)
D54	October	2000	2-Oct	04-Oct (67.62)	04-Oct (68.28)	03-Oct (83.28)	04-Oct (76.29)	04-Oct (69.56)	03-Oct (84.27)
D54	October	2400	2-Oct	05-Oct (59.18)	05-Oct (59.76)	04-Oct (72.89)	05-Oct (66.78)	05-Oct (60.88)	04-Oct (73.76)
D54	October	3000	2-Oct	08-Oct (43.89)	08-Oct (44.32)	07-Oct (54.06)	07-Oct (49.52)	08-Oct (45.15)	07-Oct (54.7)
D61	November	1400	6-Nov	07-Nov (90.52)	07-Nov (91.4)	06-Nov (111.49)	06-Nov (102.13)	07-Nov (93.12)	06-Nov (112.81)
D61	November	2000	6-Nov	11-Nov (64.71)	11-Nov (65.34)	09-Nov (79.7)	10-Nov (73.01)	11-Nov (66.57)	09-Nov (80.65)
D61	November	2400	6-Nov	12-Nov (54.41)	12-Nov (54.94)	11-Nov (67.01)	11-Nov (61.39)	12-Nov (55.97)	11-Nov (67.81)
D61	November	3000	6-Nov	16-Nov (42.36)	16-Nov (42.77)	15-Nov (52.17)	16-Nov (47.79)	16-Nov (43.57)	15-Nov (52.79)

A.2 Byrd and Butler predictions

Table A.10: Byrd and Butler TOP date mean estimation and percentage (%) ADH coverage by stage in 2013

BodyID	Month	Milestone	Actual TOP	Fluctuating 15.6° C	Fluctuating 21.1° C	Constant 25° C	Fluctuating 26.7° C	Fluctuating 32.2° C
D51	September	1400	22-Sep	19-Sep (146.04)	19-Sep (116.58)	19-Sep (103.59)	20-Sep (114.74)	19-Sep (88.95)
D51	September	2000	22-Sep	24-Sep (100.18)	23-Sep (79.96)	24-Sep (71.06)	25-Sep (78.7)	24-Sep (61.02)
D51	September	2400	22-Sep	25-Sep (81.12)	24-Sep (64.76)	26-Sep (57.54)	26-Sep (63.73)	26-Sep (49.41)
D53	October	1400	10-Oct	10-Oct (130.91)	09-Oct (104.49)	10-Oct (92.86)	10-Oct (102.84)	10-Oct (79.73)
D53	October	2000	10-Oct	11-Oct (97.02)	10-Oct (77.45)	11-Oct (68.82)	12-Oct (76.22)	11-Oct (59.09)
D53	October	2400	10-Oct	12-Oct (82.85)	11-Oct (66.14)	12-Oct (58.77)	12-Oct (65.09)	12-Oct (50.47)
D53	October	3000	10-Oct	15-Oct (62.88)	14-Oct (50.2)	15-Oct (44.6)	16-Oct (49.4)	15-Oct (38.3)
D54	October	2000	11-Oct	12-Oct (98.88)	11-Oct (78.93)	12-Oct (70.14)	12-Oct (77.68)	12-Oct (60.23)
D54	October	2400	11-Oct	13-Oct (80.62)	12-Oct (64.36)	13-Oct (57.19)	14-Oct (63.34)	14-Oct (49.11)
D54	October	3000	11-Oct	17-Oct (62.58)	15-Oct (49.95)	17-Oct (44.39)	19-Oct (49.16)	17-Oct (38.12)

Table A.11: Byrd and Butler TOP date mean estimation and percentage (%) ADH coverage by stage in 2016

BodyID	Month	Milestone	Actual TOP	Fluctuating 15.6° C	Fluctuating 21.1° C	Constant 25° C	Fluctuating 26.7° C	Fluctuating 32.2° C
D06	March	2000	19-Feb	21-Feb (94.91)	20-Feb (75.76)	22-Feb (67.32)	23-Feb (74.56)	22-Feb (57.81)
D06	March	2400	19-Feb	24-Feb (80.08)	22-Feb (63.92)	25-Feb (56.8)	27-Feb (62.91)	25-Feb (48.78)
D08	March	1400	29-Feb	25-Feb (137.85)	23-Feb (110.04)	26-Feb (97.78)	27-Feb (108.3)	26-Feb (83.96)
D08	March	2000	29-Feb	28-Feb (100.75)	25-Feb (80.42)	28-Feb (71.46)	01-Mar (79.15)	29-Feb (61.36)
D08	March	2400	29-Feb	02-Mar (80.86)	29-Feb (64.55)	03-Mar (57.36)	04-Mar (63.53)	03-Mar (49.25)
D08	March	3000	29-Feb	05-Mar (62.38)	03-Mar (49.8)	06-Mar (44.25)	07-Mar (49.01)	06-Mar (38)
D23	June	1400	16-Jun	16-Jun (138.77)	15-Jun (110.77)	16-Jun (98.43)	16-Jun (109.02)	16-Jun (84.52)
D23	June	2000	16-Jun	17-Jun (101.74)	16-Jun (81.21)	17-Jun (72.17)	17-Jun (79.93)	17-Jun (61.97)
D23	June	2400	16-Jun	18-Jun (85.49)	17-Jun (68.24)	18-Jun (60.64)	18-Jun (67.16)	18-Jun (52.07)
D23	June	3000	16-Jun	19-Jun (61.76)	19-Jun (49.3)	20-Jun (43.81)	20-Jun (48.52)	20-Jun (37.62)
D26	June	1400	21-Jun	21-Jun (147.13)	20-Jun (117.44)	21-Jun (104.36)	21-Jun (115.59)	21-Jun (89.61)
D26	June	2000	21-Jun	22-Jun (94.2)	22-Jun (75.19)	23-Jun (66.82)	23-Jun (74.01)	23-Jun (57.38)
D26	June	2400	21-Jun	23-Jun (77.99)	23-Jun (62.26)	25-Jun (55.32)	24-Jun (61.27)	24-Jun (47.5)
D26	June	3000	21-Jun	24-Jun (65.85)	24-Jun (52.56)	25-Jun (46.71)	25-Jun (51.73)	25-Jun (40.11)
D28	June	1400	21-Jun	14-Jun (142.24)	13-Jun (113.54)	14-Jun (100.89)	14-Jun (111.74)	14-Jun (86.63)
D30	June	1400	30-Jun	30-Jun (145.97)	29-Jun (116.52)	30-Jun (103.54)	30-Jun (114.68)	30-Jun (88.91)
D67	February	1400	26-Jan	25-Jan (130.85)	19-Jan (104.45)	26-Jan (92.82)	29-Jan (102.8)	28-Jan (79.7)
D67	February	2000	26-Jan	31-Jan (94.51)	29-Jan (75.44)	31-Jan (67.04)	01-Feb (74.25)	31-Jan (57.56)
D67	February	2400	26-Jan	02-Feb (80.41)	31-Jan (64.19)	04-Feb (57.04)	08-Feb (63.17)	05-Feb (48.98)

Table A.12: Byrd and Butler TOP date mean estimation and percentage (%) ADH coverage by stage in 2015

BodyID	Month	Milestone	Actual TOP	Fluctuating 15.6° C	Fluctuating 21.1° C	Constant 25° C	Fluctuating 26.7° C	Fluctuating 32.2° C
D23	June	1400	8-Jun	08-Jun (122.65)	08-Jun (97.91)	09-Jun (87)	09-Jun (96.36)	09-Jun (74.71)
D23	June	2000	8-Jun	10-Jun (94.91)	09-Jun (75.76)	10-Jun (67.32)	10-Jun (74.56)	10-Jun (57.81)
D23	June	2400	8-Jun	10-Jun (79.2)	10-Jun (62.22)	11-Jun (56.18)	11-Jun (62.22)	11-Jun (48.24)
D23	June	3000	8-Jun	12-Jun (61.14)	11-Jun (48.8)	12-Jun (43.37)	13-Jun (48.03)	12-Jun (37.24)
D24	June	1400	8-Jun	08-Jun (122.65)	07-Jun (97.9)	08-Jun (87)	09-Jun (96.36)	08-Jun (74.7)
D24	June	2000	8-Jun	09-Jun (94.91)	09-Jun (75.76)	09-Jun (67.32)	10-Jun (74.56)	09-Jun (57.81)
D24	June	2400	8-Jun	10-Jun (78.76)	09-Jun (62.87)	10-Jun (55.87)	11-Jun (61.88)	10-Jun (47.97)
D24	June	3000	8-Jun	12-Jun (61.14)	11-Jun (48.8)	12-Jun (43.37)	12-Jun (48.03)	12-Jun (37.24)
D25	June	1400	8-Jun	08-Jun (122.65)	08-Jun (97.9)	08-Jun (87)	09-Jun (96.36)	08-Jun (74.7)
D25	June	2000	8-Jun	09-Jun (96.36)	08-Jun (76.92)	09-Jun (68.35)	10-Jun (75.7)	09-Jun (58.69)
D25	June	2400	8-Jun	10-Jun (78.76)	09-Jun (62.87)	10-Jun (55.87)	11-Jun (61.88)	10-Jun (47.97)
D27	June	1400	5-Jun	05-Jun (126.13)	04-Jun (100.68)	05-Jun (89.46)	05-Jun (99.09)	05-Jun (76.82)
D27	June	2000	5-Jun	06-Jun (95.86)	05-Jun (76.52)	06-Jun (68)	07-Jun (75.31)	06-Jun (58.39)
D27	June	2400	5-Jun	07-Jun (79.6)	06-Jun (63.54)	07-Jun (56.46)	08-Jun (62.53)	07-Jun (48.48)
D27	June	3000	5-Jun	09-Jun (64.33)	08-Jun (51.35)	09-Jun (45.63)	09-Jun (50.54)	09-Jun (39.18)
D28	June	1400	4-Jun	04-Jun (128.13)	03-Jun (102.28)	04-Jun (90.89)	04-Jun (100.67)	04-Jun (78.04)
D28	June	2000	4-Jun	05-Jun (101.73)	04-Jun (81.2)	05-Jun (72.16)	05-Jun (79.92)	05-Jun (61.96)
D28	June	2400	4-Jun	06-Jun (80.68)	05-Jun (64.4)	06-Jun (57.23)	07-Jun (63.39)	06-Jun (49.14)
D30	June	1400	8-Jun	08-Jun (126.15)	08-Jun (100.7)	08-Jun (89.48)	09-Jun (99.11)	09-Jun (76.84)
D30	June	2000	8-Jun	10-Jun (96.44)	09-Jun (76.98)	10-Jun (68.41)	10-Jun (75.77)	10-Jun (58.74)
D30	June	2400	8-Jun	10-Jun (81.75)	10-Jun (65.25)	11-Jun (57.98)	11-Jun (64.22)	11-Jun (49.79)
D35	July	1400	2-Jul	01-Jul (144.97)	30-Jun (115.72)	01-Jul (102.83)	02-Jul (113.89)	01-Jul (88.3)
D35	July	2000	2-Jul	03-Jul (94.55)	03-Jul (75.47)	03-Jul (67.07)	04-Jul (74.28)	04-Jul (57.59)
D35	July	2400	2-Jul	04-Jul (77.55)	04-Jul (61.9)	05-Jul (55.01)	05-Jul (60.92)	05-Jul (47.23)
D35	July	3000	2-Jul	06-Jul (64.69)	05-Jul (51.64)	06-Jul (45.89)	06-Jul (50.82)	06-Jul (39.4)
D39	July	1400	15-Jul	15-Jul (132.83)	14-Jul (106.03)	15-Jul (94.22)	15-Jul (104.36)	15-Jul (80.91)
D39	July	2000	15-Jul	16-Jul (98.26)	15-Jul (78.44)	16-Jul (69.7)	16-Jul (77.2)	16-Jul (59.85)
D39	July	2400	15-Jul	17-Jul (80.12)	16-Jul (63.95)	17-Jul (56.83)	17-Jul (62.94)	17-Jul (48.8)
D40	August	2000	29-Jul	30-Jul (97.98)	29-Jul (78.21)	30-Jul (69.5)	31-Jul (76.97)	30-Jul (59.68)
D43	August	1400	12-Aug	12-Aug (128.57)	11-Aug (102.63)	12-Aug (91.2)	12-Aug (101.01)	12-Aug (78.31)
D43	August	2000	12-Aug	13-Aug (92.1)	12-Aug (73.52)	13-Aug (65.33)	14-Aug (72.35)	13-Aug (56.1)
D43	August	2400	12-Aug	14-Aug (79.14)	13-Aug (63.17)	14-Aug (56.13)	14-Aug (62.17)	14-Aug (48.2)
D45	September	1400	28-Aug	28-Aug (129.03)	28-Aug (103)	28-Aug (91.53)	29-Aug (101.37)	29-Aug (78.59)
D45	September	2000	28-Aug	29-Aug (100.02)	29-Aug (79.84)	30-Aug (70.95)	30-Aug (78.58)	30-Aug (60.92)
D45	September	2400	28-Aug	30-Aug (80.89)	30-Aug (64.57)	31-Aug (57.38)	31-Aug (63.55)	31-Aug (49.27)
D51	September	2000	18-Sep	19-Sep (96.18)	18-Sep (76.77)	19-Sep (68.22)	20-Sep (75.56)	19-Sep (58.58)
D51	September	2400	18-Sep	20-Sep (80.28)	19-Sep (64.08)	20-Sep (56.95)	21-Sep (63.07)	20-Sep (48.9)
D53	October	1400	2-Oct	02-Oct (128.93)	01-Oct (102.92)	02-Oct (91.46)	03-Oct (101.29)	02-Oct (78.53)
D53	October	2400	2-Oct	05-Oct (74.04)	04-Oct (59.1)	06-Oct (52.52)	06-Oct (58.17)	06-Oct (45.1)
D54	October	1400	2-Oct	02-Oct (131.86)	01-Oct (105.26)	02-Oct (93.53)	02-Oct (103.59)	02-Oct (80.31)
D54	October	2000	2-Oct	03-Oct (100.75)	02-Oct (80.42)	04-Oct (71.47)	04-Oct (79.15)	04-Oct (61.37)
D54	October	2400	2-Oct	04-Oct (88.18)	03-Oct (70.39)	04-Oct (62.55)	05-Oct (69.28)	04-Oct (53.71)
D54	October	3000	2-Oct	07-Oct (65.4)	06-Oct (52.1)	07-Oct (46.39)	07-Oct (51.38)	07-Oct (39.83)
D61	November	1400	6-Nov	06-Nov (134.87)	05-Nov (107.66)	06-Nov (95.67)	06-Nov (105.96)	06-Nov (82.15)
D61	November	2000	6-Nov	09-Nov (96.42)	07-Nov (76.96)	10-Nov (68.39)	10-Nov (75.75)	10-Nov (58.73)
D61	November	2400	6-Nov	11-Nov (86.71)	10-Nov (64.71)	11-Nov (57.51)	12-Nov (63.69)	11-Nov (49.38)
D61	November	3000	6-Nov	15-Nov (63.11)	12-Nov (50.38)	15-Nov (44.77)	16-Nov (49.58)	15-Nov (38.44)

APPENDIX B

SECOND APPENDIX - LENGTH PHENOTYPE

B.1 Boatright and Tomberlin predictions

Table B.1: Boatright and Tomberlin TOP date minimum estimation and percentage (%) ADH coverage by length in 2013. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D51	September	1400	22-Sep	20-Sep(64.64)	20-Sep(73.52)	20-Sep(61.45)
D51	September	2000	22-Sep	25-Sep(48.89)	23-Sep(54.19)	25-Sep(45.98)
D51	September	2400	22-Sep	27-Sep(25.78)	27-Sep(33.52)	27-Sep(27.93)
D53	October	1400	10-Oct	10-Oct(44.57)	15-Oct(59.02)	11-Oct(47.57)
D53	October	2000	10-Oct	19-Oct(59.46)	19-Oct(71.44)	10-Oct(56.60)
D53	October	2400	10-Oct	13-Oct(31.97)	13-Oct(39.22)	14-Oct(31.69)
D53	October	3000	10-Oct	16-Oct(34.26)	14-Oct(37.80)	18-Oct(31.27)
D54	October	2000	11-Oct	12-Oct(50.50)	21-Oct(55.72)	13-Oct(46.33)
D54	October	2400	11-Oct	14-Oct(43.92)	12-Oct(48.46)	15-Oct(40.09)
D54	October	3000	11-Oct	21-Oct(31.96)	21-Oct(35.26)	22-Oct(29.32)

Table B.2: Boatright and Tomberlin TOP date minimum estimation and percentage (%) ADH coverage by length in 2016. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D06	March	2000	19-Feb	29-Feb(39.32)	28-Feb(47.06)	29-Feb(38.12)
D06	March	2400	19-Feb	01-Mar(39.08)	29-Feb(43.32)	01-Mar(36.76)
D08	March	1400	29-Feb	01-Mar(46.94)	29-Feb(62.15)	02-Mar(50.09)
D08	March	2000	29-Feb	02-Mar(34.30)	29-Feb(45.42)	02-Mar(36.61)
D08	March	2400	29-Feb	02-Mar(39.46)	12-Mar(43.74)	05-Mar(37.12)
D08	March	3000	29-Feb	07-Mar(29.03)	03-Mar(32.34)	08-Mar(27.44)
D23	June	1400	16-Jun	16-Jun(77.17)	19-Jun(87.58)	17-Jun(71.66)
D23	June	2000	16-Jun	18-Jun(34.64)	17-Jun(45.87)	18-Jun(36.97)
D23	June	2400	16-Jun	19-Jun(26.20)	18-Jun(32.12)	19-Jun(26.16)
D23	June	3000	16-Jun	21-Jun(31.54)	21-Jun(34.80)	21-Jun(28.94)
D26	June	1400	21-Jun	22-Jun(45.08)	22-Jun(55.27)	22-Jun(45.02)
D26	June	2000	21-Jun	24-Jun(15.50)	24-Jun(24.06)	24-Jun(14.41)
D26	June	2400	21-Jun	24-Jun(32.31)	24-Jun(38.68)	25-Jun(31.33)
D26	June	3000	21-Jun	26-Jun(25.41)	25-Jun(31.17)	26-Jun(25.19)
D28	June	1400	21-Jun	15-Jun(79.10)	15-Jun(89.77)	15-Jun(73.45)
D30	June	1400	30-Jun	01-Jul(60.47)	30-Jun(72.39)	01-Jul(58.65)
D67	February	1400	26-Jan	29-Jan(72.77)	19-Jan(82.59)	31-Jan(67.57)
D67	February	2000	26-Jan	15-Feb(66.50)	15-Feb(74.56)	29-Jan(58.74)
D67	February	2400	26-Jan	18-Feb(58.41)	NA	31-Jan(51.52)

Table B.3: Boatright and Tomberlin TOP date minimum estimation and percentage (%) ADH coverage by length in 2015. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D23	June	1400	8-Jun	10-Jun(50.81)	09-Jun(60.82)	10-Jun(49.26)
D23	June	2000	8-Jun	11-Jun(30.16)	10-Jun(39.22)	11-Jun(32.67)
D23	June	2400	8-Jun	11-Jun(32.81)	11-Jun(39.28)	11-Jun(31.81)
D23	June	3000	8-Jun	13-Jun(31.22)	13-Jun(34.45)	13-Jun(28.65)
D24	June	1400	8-Jun	09-Jun(50.81)	09-Jun(60.82)	10-Jun(49.26)
D24	June	2000	8-Jun	11-Jun(36.62)	10-Jun(44.92)	11-Jun(36.30)
D24	June	2400	8-Jun	11-Jun(36.65)	11-Jun(40.83)	12-Jun(34.65)
D24	June	3000	8-Jun	13-Jun(33.31)	12-Jun(36.75)	13-Jun(30.40)
D25	June	1400	8-Jun	09-Jun(57.07)	09-Jun(63.59)	09-Jun(53.95)
D25	June	2000	8-Jun	10-Jun(47.03)	10-Jun(52.13)	11-Jun(44.23)
D25	June	2400	8-Jun	14-Jun(40.23)	14-Jun(44.38)	10-Jun(36.91)
D27	June	1400	5-Jun	05-Jun(48.67)	09-Jun(59.70)	06-Jun(48.24)
D27	June	2000	5-Jun	07-Jun(39.71)	07-Jun(47.54)	08-Jun(38.50)
D27	June	2400	5-Jun	07-Jun(38.85)	11-Jun(43.06)	08-Jun(36.54)
D27	June	3000	5-Jun	09-Jun(37.24)	08-Jun(43.02)	10-Jun(34.45)
D28	June	1400	4-Jun	06-Jun(8.73)	05-Jun(11.55)	NA
D28	June	2000	4-Jun	07-Jun(25.40)	06-Jun(31.34)	07-Jun(27.24)
D28	June	2400	4-Jun	08-Jun(33.42)	08-Jun(40.01)	08-Jun(32.41)
D30	June	1400	8-Jun	09-Jun(58.70)	09-Jun(65.40)	10-Jun(55.49)
D30	June	2000	8-Jun	11-Jun(37.21)	10-Jun(45.65)	11-Jun(36.89)
D30	June	2400	8-Jun	11-Jun(33.86)	11-Jun(40.54)	11-Jun(32.83)
D35	July	1400	2-Jul	03-Jul(46.07)	02-Jul(59.91)	03-Jul(49.91)
D35	July	2000	2-Jul	05-Jul(28.97)	05-Jul(35.52)	05-Jul(28.93)
D35	July	2400	2-Jul	06-Jul(24.64)	06-Jul(32.05)	06-Jul(26.70)
D35	July	3000	2-Jul	07-Jul(31.57)	07-Jul(35.00)	07-Jul(29.69)
D39	July	1400	15-Jul	15-Jul(51.26)	14-Jul(62.88)	16-Jul(50.81)
D39	July	2000	15-Jul	17-Jul(31.23)	17-Jul(40.61)	17-Jul(33.83)
D39	July	2400	15-Jul	18-Jul(24.55)	18-Jul(30.10)	18-Jul(24.52)
D40	August	2000	29-Jul	31-Jul(19.46)	31-Jul(27.24)	01-Aug(17.80)
D43	August	1400	12-Aug	13-Aug(71.50)	12-Aug(81.15)	13-Aug(66.39)
D43	August	2000	12-Aug	16-Aug(64.81)	16-Aug(72.66)	12-Aug(57.25)
D43	August	2400	12-Aug	15-Aug(15.72)	15-Aug(22.00)	15-Aug(14.38)
D45	September	1400	28-Aug	01-Sep(62.97)	01-Sep(69.80)	28-Aug(59.23)
D45	September	2000	28-Aug	30-Aug(30.65)	29-Aug(37.58)	30-Aug(30.61)
D45	September	2400	28-Aug	31-Aug(24.79)	31-Aug(30.39)	01-Sep(24.75)
D51	September	2000	18-Sep	20-Sep(37.11)	20-Sep(45.55)	21-Sep(36.79)
D51	September	2400	18-Sep	21-Sep(39.18)	21-Sep(43.43)	21-Sep(36.85)
D53	October	1400	2-Oct	02-Oct(71.70)	07-Oct(81.38)	03-Oct(66.58)
D53	October	2400	2-Oct	06-Oct(40.33)	04-Oct(44.51)	07-Oct(36.82)
D54	October	1400	2-Oct	02-Oct(58.36)	01-Oct(66.38)	03-Oct(55.48)
D54	October	2000	2-Oct	05-Oct(46.88)	04-Oct(52.23)	05-Oct(44.32)
D54	October	2400	2-Oct	06-Oct(34.03)	05-Oct(41.74)	06-Oct(33.73)
D54	October	3000	2-Oct	11-Oct(31.92)	11-Oct(35.38)	06-Oct(30.02)
D61	November	1400	6-Nov	09-Nov(38.27)	09-Nov(47.63)	10-Nov(38.69)
D61	November	2000	6-Nov	11-Nov(37.21)	10-Nov(45.64)	11-Nov(36.88)
D61	November	2400	6-Nov	13-Nov(23.00)	12-Nov(28.63)	14-Nov(23.26)
D61	November	3000	6-Nov	19-Nov(27.94)	18-Nov(31.77)	19-Nov(26.56)

Table B.4: Boatright and Tomberlin TOP date mean estimation and percentage (%) ADH coverage by length in 2013. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C min	Pork/Equine 20.8° C max	Pork/Equine 24.3° C min	Pork/Equine 24.3° C max	Pork/Equine 28.2° C min	Pork/Equine 28.2° C max
D51	September	1400	22-Sep	21-Sep(74.59)	21-Sep(74.59)	21-Sep(62.16)	21-Sep(62.16)	22-Sep(40.61)	22-Sep(40.61)
D51	September	2000	22-Sep	26-Sep(55.71)	26-Sep(55.71)	26-Sep(47.75)	26-Sep(47.75)	27-Sep(30.70)	27-Sep(30.70)
D51	September	2400	22-Sep	28-Sep(33.61)	28-Sep(33.61)	28-Sep(30.38)	28-Sep(30.38)	29-Sep(19.33)	29-Sep(19.33)
D53	October	1400	10-Oct	11-Oct(66.86)	11-Oct(66.86)	12-Oct(55.71)	12-Oct(55.71)	13-Oct(36.40)	13-Oct(36.40)
D53	October	2000	10-Oct	NA	NA	NA	NA	13-Oct(46.25)	13-Oct(46.25)
D53	October	2400	10-Oct	14-Oct(38.55)	14-Oct(38.55)	15-Oct(32.44)	15-Oct(32.44)	16-Oct(21.63)	16-Oct(21.63)
D53	October	3000	10-Oct	18-Oct(36.40)	18-Oct(36.40)	20-Oct(31.76)	20-Oct(31.76)	22-Oct(19.98)	22-Oct(19.98)
D54	October	2000	11-Oct	13-Oct(58.36)	13-Oct(58.36)	14-Oct(52.75)	14-Oct(52.75)	15-Oct(31.98)	15-Oct(31.98)
D54	October	2400	11-Oct	15-Oct(47.58)	15-Oct(47.58)	15-Oct(43.01)	15-Oct(43.01)	19-Oct(26.08)	19-Oct(26.08)
D54	October	3000	11-Oct	21-Oct(31.96)	21-Oct(31.96)	23-Oct(26.63)	23-Oct(26.63)	24-Oct(17.40)	24-Oct(17.40)

Table B.5: Boatright and Tomberlin TOP date mean estimation and percentage (%) ADH coverage by length in 2016. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C min	Pork/Equine 20.8° C max	Pork/Equine 24.3° C min	Pork/Equine 24.3° C max	Pork/Equine 28.2° C min	Pork/Equine 28.2° C max
D06	March	2400	19-Feb	01-Mar(39.08)	01-Mar(39.08)	01-Mar(32.72)	01-Mar(32.72)	03-Mar(21.81)	03-Mar(21.81)
D08	March	1400	29-Feb	02-Mar(61.02)	02-Mar(61.02)	03-Mar(52.41)	03-Mar(52.41)	04-Mar(34.42)	04-Mar(34.42)
D08	March	2000	29-Feb	04-Mar(49.17)	04-Mar(49.17)	04-Mar(41.16)	04-Mar(41.16)	06-Mar(27.44)	06-Mar(27.44)
D08	March	2400	29-Feb	06-Mar(46.80)	06-Mar(46.80)	07-Mar(40.84)	07-Mar(40.84)	09-Mar(25.70)	09-Mar(25.70)
D23	June	1400	16-Jun	09-Mar(34.69)	09-Mar(34.69)	10-Mar(29.74)	10-Mar(29.74)	12-Mar(19.12)	12-Mar(19.12)
D23	June	2000	16-Jun	17-Jun(81.89)	17-Jun(81.89)	17-Jun(74.02)	17-Jun(74.02)	18-Jun(44.88)	18-Jun(44.88)
D23	June	2400	16-Jun	18-Jun(49.65)	18-Jun(49.65)	19-Jun(41.57)	19-Jun(41.57)	19-Jun(27.71)	19-Jun(27.71)
D23	June	3000	16-Jun	19-Jun(37.84)	19-Jun(37.84)	20-Jun(32.50)	20-Jun(32.50)	20-Jun(21.34)	20-Jun(21.34)
D26	June	1400	21-Jun	21-Jun(31.54)	21-Jun(31.54)	21-Jun(26.29)	21-Jun(26.29)	22-Jun(17.17)	22-Jun(17.17)
D26	June	2000	21-Jun	23-Jun(60.95)	23-Jun(60.95)	23-Jun(55.10)	23-Jun(55.10)	23-Jun(35.07)	23-Jun(35.07)
D26	June	2400	21-Jun	25-Jun(28.87)	25-Jun(28.87)	25-Jun(26.73)	25-Jun(26.73)	25-Jun(17.11)	25-Jun(17.11)
D26	June	3000	21-Jun	25-Jun(39.83)	25-Jun(39.83)	25-Jun(33.19)	25-Jun(33.19)	26-Jun(21.69)	26-Jun(21.69)
D28	June	1400	21-Jun	26-Jun(32.14)	26-Jun(32.14)	26-Jun(26.90)	26-Jun(26.90)	27-Jun(17.94)	27-Jun(17.94)
D30	June	1400	30-Jun	15-Jun(79.10)	15-Jun(79.10)	15-Jun(67.80)	15-Jun(67.80)	16-Jun(43.59)	16-Jun(43.59)
D67	February	1400	26-Jan	01-Jul(74.55)	01-Jul(74.55)	01-Jul(62.12)	01-Jul(62.12)	02-Jul(40.59)	02-Jul(40.59)
D67	February	2000	26-Jan	31-Jan(77.22)	31-Jan(77.22)	31-Jan(69.80)	31-Jan(69.80)	05-Feb(42.32)	05-Feb(42.32)
D67	February	2400	26-Jan	01-Feb(70.79)	01-Feb(70.79)	NA	NA	11-Feb(37.54)	11-Feb(37.54)
				NA	NA	NA	NA	12-Feb(38.33)	12-Feb(38.33)

Table B.6: Boatright and Tomberlin TOP date mean estimation and percentage (%) ADH coverage by length in 2015. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C min	Pork/Equine 20.8° C max	Pork/Equine 24.3° C min	Pork/Equine 24.3° C max	Pork/Equine 28.2° C min	Pork/Equine 28.2° C max
D23	June	1400	8-Jun	10-Jun(62.64)	10-Jun(62.64)	10-Jun(52.20)	10-Jun(52.20)	11-Jun(34.10)	11-Jun(34.10)
D23	June	2000	8-Jun	11-Jun(44.16)	11-Jun(44.16)	12-Jun(37.16)	12-Jun(37.16)	12-Jun(24.77)	12-Jun(24.77)
D23	June	2400	8-Jun	12-Jun(43.15)	12-Jun(43.15)	12-Jun(35.96)	12-Jun(35.96)	13-Jun(23.37)	13-Jun(23.37)
D23	June	3000	8-Jun	13-Jun(33.31)	13-Jun(33.31)	14-Jun(27.75)	14-Jun(27.75)	15-Jun(18.04)	15-Jun(18.04)
D24	June	1400	8-Jun	10-Jun(59.86)	10-Jun(59.86)	10-Jun(50.11)	10-Jun(50.11)	11-Jun(33.41)	11-Jun(33.41)
D24	June	2000	8-Jun	11-Jun(42.01)	11-Jun(42.01)	11-Jun(36.08)	11-Jun(36.08)	12-Jun(23.70)	12-Jun(23.70)
D24	June	2400	8-Jun	12-Jun(40.23)	12-Jun(40.23)	12-Jun(33.52)	12-Jun(33.52)	13-Jun(21.90)	13-Jun(21.90)
D24	June	3000	8-Jun	13-Jun(35.39)	13-Jun(35.39)	13-Jun(30.88)	13-Jun(30.88)	14-Jun(19.43)	14-Jun(19.43)
D25	June	1400	8-Jun	09-Jun(66.82)	09-Jun(66.82)	10-Jun(55.68)	10-Jun(55.68)	11-Jun(36.19)	11-Jun(36.19)
D25	June	2000	8-Jun	10-Jun(53.59)	10-Jun(53.59)	11-Jun(45.93)	11-Jun(45.93)	12-Jun(29.53)	12-Jun(29.53)
D25	June	2400	8-Jun	11-Jun(48.27)	11-Jun(48.27)	12-Jun(43.80)	12-Jun(43.80)	12-Jun(27.26)	12-Jun(27.26)
D27	June	1400	5-Jun	06-Jun(68.71)	06-Jun(68.71)	07-Jun(57.26)	07-Jun(57.26)	08-Jun(37.22)	08-Jun(37.22)
D27	June	2000	5-Jun	08-Jun(46.78)	08-Jun(46.78)	08-Jun(39.17)	08-Jun(39.17)	09-Jun(26.11)	09-Jun(26.11)
D27	June	2400	5-Jun	08-Jun(46.07)	08-Jun(46.07)	09-Jun(40.20)	09-Jun(40.20)	10-Jun(25.29)	10-Jun(25.29)
D27	June	3000	5-Jun	10-Jun(37.97)	10-Jun(37.97)	10-Jun(34.32)	10-Jun(34.32)	11-Jun(20.81)	11-Jun(20.81)
D28	June	1400	4-Jun	07-Jun(36.36)	07-Jun(36.36)	07-Jun(34.17)	07-Jun(34.17)	07-Jun(21.81)	07-Jun(21.81)
D28	June	2000	4-Jun	07-Jun(34.64)	07-Jun(34.64)	07-Jun(34.64)	07-Jun(34.64)	08-Jun(21.94)	08-Jun(21.94)
D28	June	2400	4-Jun	08-Jun(39.37)	08-Jun(39.37)	08-Jun(32.96)	08-Jun(32.96)	09-Jun(21.98)	09-Jun(21.98)
D30	June	1400	8-Jun	10-Jun(68.72)	10-Jun(68.72)	10-Jun(57.27)	10-Jun(57.27)	11-Jun(37.23)	11-Jun(37.23)
D30	June	2000	8-Jun	11-Jun(49.25)	11-Jun(49.25)	11-Jun(41.04)	11-Jun(41.04)	12-Jun(26.82)	12-Jun(26.82)
D30	June	2400	8-Jun	12-Jun(44.53)	12-Jun(44.53)	12-Jun(37.11)	12-Jun(37.11)	13-Jun(24.12)	13-Jun(24.12)
D35	July	1400	2-Jul	03-Jul(60.05)	03-Jul(60.05)	03-Jul(54.29)	03-Jul(54.29)	04-Jul(34.55)	04-Jul(34.55)
D35	July	2000	2-Jul	05-Jul(39.17)	05-Jul(39.17)	05-Jul(35.41)	05-Jul(35.41)	06-Jul(22.54)	06-Jul(22.54)
D35	July	2400	2-Jul	06-Jul(32.12)	06-Jul(32.12)	06-Jul(29.04)	06-Jul(29.04)	07-Jul(18.48)	07-Jul(18.48)
D35	July	3000	2-Jul	07-Jul(31.57)	07-Jul(31.57)	08-Jul(26.43)	08-Jul(26.43)	08-Jul(17.62)	08-Jul(17.62)
D39	July	1400	15-Jul	16-Jul(72.36)	16-Jul(72.36)	16-Jul(60.30)	16-Jul(60.30)	17-Jul(39.20)	17-Jul(39.20)
D39	July	2000	15-Jul	18-Jul(43.49)	18-Jul(43.49)	18-Jul(37.36)	18-Jul(37.36)	18-Jul(24.53)	18-Jul(24.53)
D39	July	2400	15-Jul	19-Jul(33.19)	19-Jul(33.19)	19-Jul(30.01)	19-Jul(30.01)	19-Jul(19.09)	19-Jul(19.09)
D40	August	2000	29-Jul	01-Aug(33.36)	01-Aug(33.36)	01-Aug(33.36)	01-Aug(33.36)	02-Aug(21.13)	02-Aug(21.13)
D43	August	1400	12-Aug	13-Aug(75.88)	13-Aug(75.88)	13-Aug(68.58)	13-Aug(68.58)	14-Aug(41.59)	14-Aug(41.59)
D43	August	2000	12-Aug	13-Aug(81.53)	13-Aug(81.53)	NA	NA	15-Aug(36.58)	15-Aug(36.58)
D43	August	2400	12-Aug	16-Aug(25.15)	16-Aug(25.15)	16-Aug(16.17)	16-Aug(16.17)	16-Aug(16.17)	16-Aug(16.17)
D43	August	3000	12-Aug	29-Aug(76.15)	29-Aug(76.15)	30-Aug(68.83)	30-Aug(68.83)	31-Aug(41.74)	31-Aug(41.74)
D45	September	1400	28-Aug	31-Aug(48.81)	31-Aug(48.81)	31-Aug(40.87)	31-Aug(40.87)	01-Sep(27.24)	01-Sep(27.24)
D45	September	2000	28-Aug	01-Sep(37.64)	01-Sep(37.64)	01-Sep(31.67)	01-Sep(31.67)	02-Sep(21.11)	02-Sep(21.11)
D51	September	2400	18-Sep	21-Sep(46.94)	21-Sep(46.94)	21-Sep(39.30)	21-Sep(39.30)	22-Sep(26.20)	22-Sep(26.20)
D51	September	3000	18-Sep	21-Sep(44.65)	21-Sep(44.65)	22-Sep(38.27)	22-Sep(38.27)	23-Sep(24.60)	23-Sep(24.60)
D53	October	1400	2-Oct	03-Oct(76.09)	03-Oct(76.09)	04-Oct(68.78)	04-Oct(68.78)	05-Oct(41.70)	05-Oct(41.70)
D53	October	2000	2-Oct	07-Oct(43.70)	07-Oct(43.70)	07-Oct(39.49)	07-Oct(39.49)	08-Oct(23.95)	08-Oct(23.95)
D54	October	1400	2-Oct	04-Oct(73.33)	04-Oct(73.33)	04-Oct(62.85)	04-Oct(62.85)	05-Oct(40.41)	05-Oct(40.41)
D54	October	2000	2-Oct	05-Oct(56.03)	05-Oct(56.03)	06-Oct(48.02)	06-Oct(48.02)	07-Oct(30.87)	07-Oct(30.87)
D54	October	2400	2-Oct	06-Oct(43.04)	06-Oct(43.04)	07-Oct(36.03)	07-Oct(36.03)	08-Oct(24.02)	08-Oct(24.02)
D54	October	3000	2-Oct	08-Oct(38.60)	08-Oct(38.60)	08-Oct(34.89)	08-Oct(34.89)	10-Oct(21.15)	10-Oct(21.15)
D61	November	1400	6-Nov	11-Nov(52.04)	11-Nov(52.04)	11-Nov(48.22)	11-Nov(48.22)	11-Nov(30.61)	11-Nov(30.61)
D61	November	2000	6-Nov	12-Nov(47.05)	12-Nov(47.05)	13-Nov(39.39)	13-Nov(39.39)	15-Nov(26.26)	15-Nov(26.26)
D61	November	2400	6-Nov	15-Nov(35.88)	15-Nov(35.88)	16-Nov(30.82)	16-Nov(30.82)	17-Nov(20.24)	17-Nov(20.24)
D61	November	3000	6-Nov	19-Nov(27.94)	19-Nov(27.94)	20-Nov(24.00)	20-Nov(24.00)	21-Nov(15.76)	21-Nov(15.76)

Table B.7: Boatright and Tomberlin TOP date maximum estimation and percentage (%) ADH coverage by length in 2013. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D51	September	1400	22-Sep	21-Sep(86.19)	20-Sep(103.15)	21-Sep(79.61)
D51	September	2000	22-Sep	26-Sep(70.49)	25-Sep(100.11)	26-Sep(62.27)
D51	September	2400	22-Sep	28-Sep(39.59)	27-Sep(43.89)	28-Sep(37.24)
D53	October	1400	10-Oct	12-Oct(109.94)	NA	12-Oct(83.87)
D53	October	2000	10-Oct	NA	NA	12-Oct(98.35)
D53	October	2400	10-Oct	15-Oct(46.08)	13-Oct(52.29)	14-Oct(42.79)
D53	October	3000	10-Oct	19-Oct(44.25)	16-Oct(62.84)	18-Oct(39.09)
D54	October	2000	11-Oct	14-Oct(87.53)	NA	14-Oct(66.19)
D54	October	2400	11-Oct	15-Oct(56.73)	14-Oct(80.57)	15-Oct(50.11)
D54	October	3000	11-Oct	21-Oct(31.96)	21-Oct(35.26)	21-Oct(29.32)

Table B.8: Boatright and Tomberlin TOP date maximum estimation and percentage (%) ADH coverage by length in 2016. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D06	March	2000	19-Feb	29-Feb(39.32)	29-Feb(47.06)	28-Feb(38.12)
D06	March	2400	19-Feb	01-Mar(39.08)	01-Mar(43.32)	29-Feb(36.76)
D08	March	1400	29-Feb	03-Mar(67.27)	01-Mar(74.57)	02-Mar(63.28)
D08	March	2000	29-Feb	05-Mar(61.74)	02-Mar(74.18)	04-Mar(58.77)
D08	March	2400	29-Feb	07-Mar(67.91)	NA	06-Mar(51.81)
D23	June	1400	29-Feb	10-Mar(43.90)	07-Mar(62.34)	09-Mar(38.78)
D23	June	2000	16-Jun	17-Jun(116.54)	NA	17-Jun(88.91)
D23	June	2400	16-Jun	19-Jun(60.04)	18-Jun(71.86)	19-Jun(55.46)
D23	June	3000	16-Jun	20-Jun(47.54)	19-Jun(53.95)	20-Jun(44.14)
D26	June	1400	21-Jun	21-Jun(31.54)	21-Jun(34.80)	21-Jun(28.94)
D26	June	2000	21-Jun	23-Jun(80.15)	22-Jun(88.44)	23-Jun(73.16)
D26	June	2400	21-Jun	25-Jun(41.70)	24-Jun(47.42)	25-Jun(39.64)
D26	June	3000	21-Jun	25-Jun(46.03)	24-Jun(55.09)	25-Jun(42.51)
D28	June	1400	21-Jun	27-Jun(38.11)	26-Jun(44.03)	26-Jun(35.26)
D30	June	1400	21-Jun	15-Jun(79.10)	15-Jun(89.77)	15-Jun(73.45)
D67	February	1400	30-Jun	01-Jul(84.49)	01-Jul(97.61)	01-Jul(78.17)
D67	February	2000	26-Jan	31-Jan(92.07)	29-Jan(130.76)	30-Jan(81.33)
D67	February	2400	26-Jan	NA	NA	31-Jan(92.18)
D67	February	2400	26-Jan	NA	NA	18-Feb(81.51)

Table B.9: Boatright and Tomberlin TOP date maximum estimation and percentage (%) ADH coverage by length in 2015. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D23	June	1400	8-Jun	10-Jun(72.39)	10-Jun(86.63)	10-Jun(66.86)
D23	June	2000	8-Jun	12-Jun(52.78)	11-Jun(59.90)	11-Jun(49.01)
D23	June	2400	8-Jun	12-Jun(48.54)	11-Jun(58.32)	12-Jun(46.20)
D23	June	3000	8-Jun	14-Jun(35.39)	13-Jun(40.88)	13-Jun(32.74)
D24	June	1400	8-Jun	10-Jun(68.21)	09-Jun(77.41)	10-Jun(63.34)
D24	June	2000	8-Jun	11-Jun(48.47)	11-Jun(53.48)	11-Jun(44.47)
D24	June	2400	8-Jun	12-Jun(43.80)	11-Jun(49.71)	12-Jun(40.67)
D24	June	3000	8-Jun	13-Jun(36.08)	13-Jun(43.18)	13-Jun(33.32)
D25	June	1400	8-Jun	10-Jun(72.38)	09-Jun(86.63)	10-Jun(66.86)
D25	June	2000	8-Jun	11-Jun(56.87)	10-Jun(68.06)	11-Jun(52.52)
D25	June	2400	8-Jun	NA	NA	11-Jun(76.83)
D27	June	1400	5-Jun	07-Jun(105.93)	NA	07-Jun(80.81)
D27	June	2000	5-Jun	08-Jun(55.49)	07-Jun(64.11)	08-Jun(51.34)
D27	June	2400	5-Jun	09-Jun(70.46)	NA	09-Jun(53.28)
D27	June	3000	5-Jun	10-Jun(45.27)	09-Jun(64.29)	09-Jun(39.99)
D28	June	1400	4-Jun	08-Jun(56.72)	06-Jun(64.51)	08-Jun(53.91)
D28	June	2000	4-Jun	08-Jun(47.34)	07-Jun(52.74)	07-Jun(44.75)
D28	June	2400	4-Jun	08-Jun(43.95)	08-Jun(48.50)	08-Jun(40.12)
D30	June	1400	8-Jun	10-Jun(74.45)	09-Jun(89.10)	10-Jun(68.76)
D30	June	2000	8-Jun	12-Jun(56.92)	11-Jun(68.11)	11-Jun(52.57)
D30	June	2400	8-Jun	12-Jun(50.10)	11-Jun(60.19)	12-Jun(47.68)
D35	July	1400	2-Jul	03-Jul(70.75)	03-Jul(78.43)	03-Jul(66.54)
D35	July	2000	2-Jul	06-Jul(48.29)	05-Jul(53.28)	05-Jul(44.31)
D35	July	2400	2-Jul	07-Jul(37.84)	06-Jul(41.95)	06-Jul(35.60)
D35	July	3000	2-Jul	07-Jul(31.57)	07-Jul(35.00)	07-Jul(29.69)
D39	July	1400	15-Jul	17-Jul(93.47)	15-Jul(132.74)	16-Jul(82.57)
D39	July	2000	15-Jul	18-Jul(50.18)	17-Jul(55.37)	18-Jul(46.04)
D39	July	2400	15-Jul	19-Jul(40.92)	18-Jul(45.15)	19-Jul(37.54)
D40	August	2000	29-Jul	02-Aug(47.82)	31-Jul(53.00)	01-Aug(44.97)
D43	August	1400	12-Aug	13-Aug(78.80)	13-Aug(94.67)	13-Aug(75.00)
D43	August	2000	12-Aug	NA	NA	13-Aug(90.71)
D43	August	2400	12-Aug	16-Aug(36.82)	15-Aug(41.03)	16-Aug(34.81)
D45	September	1400	28-Aug	NA	NA	30-Aug(125.86)
D45	September	2000	28-Aug	01-Sep(70.38)	30-Aug(99.95)	31-Aug(62.17)
D45	September	2400	28-Aug	02-Sep(49.57)	31-Aug(59.56)	02-Sep(47.18)
D51	September	2000	18-Sep	21-Sep(53.49)	20-Sep(60.70)	21-Sep(49.67)
D51	September	2400	18-Sep	22-Sep(47.38)	21-Sep(56.70)	21-Sep(43.76)
D53	October	1400	2-Oct	04-Oct(108.28)	NA	03-Oct(82.61)
D53	October	2000	2-Oct	07-Oct(52.10)	06-Oct(73.99)	07-Oct(46.02)
D53	October	2400	2-Oct	04-Oct(92.78)	02-Oct(131.77)	04-Oct(81.96)
D54	October	1400	2-Oct	06-Oct(61.75)	05-Oct(58.77)	05-Oct(46.77)
D54	October	2000	2-Oct	07-Oct(51.04)	06-Oct(58.97)	06-Oct(47.22)
D54	October	2400	2-Oct	NA	NA	08-Oct(61.29)
D54	October	3000	2-Oct	11-Nov(68.88)	09-Nov(76.00)	11-Nov(63.20)
D61	November	1400	6-Nov	13-Nov(56.90)	11-Nov(68.10)	12-Nov(52.56)
D61	November	2000	6-Nov	17-Nov(46.93)	13-Nov(54.21)	16-Nov(43.42)
D61	November	2400	6-Nov	19-Nov(27.94)	19-Nov(31.77)	18-Nov(26.56)

B.2 Byrd and Butler predictions

Table B.10: Byrd and Butler TOP date minimum estimation and percentage (%) ADH coverage by length in 2013. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Fluctuating 15.6° C	Fluctuating 21.1° C	Constant 25° C	Fluctuating 26.7° C	Fluctuating 32.2° C
D51	September	1400	22-Sep	19-Sep(43.06)	19-Sep(46.14)	19-Sep(43.64)	19-Sep(48.59)	19-Sep(40.37)
D51	September	2000	22-Sep	25-Sep(37.01)	23-Sep(39.97)	24-Sep(37.49)	23-Sep(41.74)	22-Sep(33.86)
D51	September	2400	22-Sep	26-Sep(23.94)	25-Sep(25.91)	25-Sep(25.65)	25-Sep(25.81)	24-Sep(22.63)
D53	October	1400	10-Oct	15-Oct(60.16)	10-Oct(63.03)	10-Oct(61.00)	10-Oct(60.23)	09-Oct(56.22)
D53	October	2000	10-Oct	19-Oct(62.49)	19-Oct(57.26)	19-Oct(56.85)	19-Oct(59.77)	11-Oct(51.41)
D53	October	2400	10-Oct	12-Oct(34.85)	11-Oct(36.90)	11-Oct(34.53)	11-Oct(37.02)	10-Oct(32.17)
D53	October	3000	10-Oct	17-Oct(67.02)	14-Oct(70.24)	14-Oct(61.90)	14-Oct(68.92)	13-Oct(56.50)
D54	October	2000	11-Oct	21-Oct(49.10)	12-Oct(48.66)	12-Oct(45.12)	11-Oct(50.23)	12-Oct(40.74)
D54	October	2400	11-Oct	14-Oct(42.91)	12-Oct(44.46)	13-Oct(39.83)	12-Oct(44.35)	12-Oct(38.66)
D54	October	3000	11-Oct	17-Oct(32.19)	15-Oct(32.27)	15-Oct(29.24)	15-Oct(32.56)	14-Oct(26.41)

Table B.11: Byrd and Butler TOP date minimum estimation and percentage (%) ADH coverage by length in 2016. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Fluctuating 15.6° C	Fluctuating 21.1° C	Constant 25° C	Fluctuating 26.7° C	Fluctuating 32.2° C
D06	March	2000	19-Feb	22-Feb(28.48)	20-Feb(30.34)	21-Feb(28.03)	20-Feb(31.20)	19-Feb(26.88)
D06	March	2400	19-Feb	25-Feb(65.29)	22-Feb(70.51)	23-Feb(66.14)	22-Feb(73.64)	21-Feb(59.73)
D08	March	1400	29-Feb	26-Feb(61.85)	24-Feb(63.00)	25-Feb(62.12)	24-Feb(61.48)	22-Feb(56.19)
D08	March	2000	29-Feb	01-Mar(33.90)	26-Feb(56.47)	27-Feb(54.66)	26-Feb(53.96)	25-Feb(50.37)
D08	March	2400	29-Feb	12-Mar(43.10)	02-Mar(46.85)	03-Mar(43.20)	01-Mar(48.10)	29-Feb(39.69)
D08	March	3000	29-Feb	07-Mar(35.96)	04-Mar(38.46)	05-Mar(36.13)	04-Mar(40.22)	03-Mar(33.77)
D23	June	1400	16-Jun	19-Jun(52.77)	16-Jun(54.06)	16-Jun(49.23)	15-Jun(55.69)	15-Jun(47.01)
D23	June	2000	16-Jun	17-Jun(33.31)	16-Jun(34.90)	17-Jun(33.78)	16-Jun(33.35)	16-Jun(31.13)
D23	June	2400	16-Jun	18-Jun(23.50)	17-Jun(24.01)	17-Jun(25.57)	17-Jun(25.18)	17-Jun(22.56)
D23	June	3000	16-Jun	20-Jun(71.05)	19-Jun(71.22)	19-Jun(64.53)	19-Jun(71.84)	18-Jun(58.27)
D26	June	1400	21-Jun	21-Jun(38.02)	20-Jun(36.79)	21-Jun(41.70)	20-Jun(39.28)	20-Jun(35.60)
D26	June	2000	21-Jun	23-Jun(25.15)	22-Jun(23.43)	22-Jun(24.26)	22-Jun(26.25)	21-Jun(21.94)
D26	June	2400	21-Jun	24-Jun(26.90)	23-Jun(28.82)	23-Jun(27.26)	23-Jun(30.35)	22-Jun(25.21)
D26	June	3000	21-Jun	25-Jun(63.20)	24-Jun(66.93)	24-Jun(62.62)	24-Jun(67.13)	23-Jun(58.35)
D28	June	1400	21-Jun	14-Jun(49.34)	13-Jun(50.54)	14-Jun(46.03)	13-Jun(52.07)	13-Jun(43.95)
D30	June	1400	30-Jun	30-Jun(33.96)	29-Jun(36.39)	29-Jun(34.42)	29-Jun(38.32)	28-Jun(31.84)
D67	February	1400	26-Jan	12-Feb(49.71)	25-Jan(50.92)	25-Jan(46.38)	21-Jan(52.47)	18-Jan(46.50)
D67	February	2000	26-Jan	NA	NA	31-Jan(53.02)	31-Jan(47.78)	31-Jan(41.10)
D67	February	2400	26-Jan	NA	18-Feb(88.44)	18-Feb(91.26)	18-Feb(82.25)	05-Feb(72.36)

Table B.12: Byrd and Butler TOP date minimum estimation and percentage (%) ADH coverage by length in 2015. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Fluctuating 15.6° C	Fluctuating 21.1° C	Constant 25° C	Fluctuating 26.7° C	Fluctuating 32.2° C
D23	June	1400	8-Jun	09-Jun(54.55)	08-Jun(58.11)	08-Jun(53.68)	08-Jun(59.76)	07-Jun(51.49)
D23	June	2000	8-Jun	10-Jun(37.23)	09-Jun(40.26)	09-Jun(40.04)	09-Jun(39.54)	08-Jun(35.78)
D23	June	2400	8-Jun	11-Jun(34.43)	10-Jun(36.67)	10-Jun(33.88)	10-Jun(37.72)	09-Jun(32.50)
D23	June	3000	8-Jun	12-Jun(32.56)	12-Jun(32.27)	12-Jun(29.92)	11-Jun(33.31)	11-Jun(27.02)
D24	June	1400	8-Jun	08-Jun(55.42)	07-Jun(59.04)	08-Jun(54.53)	07-Jun(60.71)	07-Jun(52.31)
D24	June	2000	8-Jun	09-Jun(43.70)	09-Jun(46.28)	09-Jun(43.30)	09-Jun(46.42)	08-Jun(40.35)
D24	June	2400	8-Jun	10-Jun(36.80)	10-Jun(38.59)	10-Jun(36.88)	10-Jun(41.06)	09-Jun(33.88)
D24	June	3000	8-Jun	12-Jun(64.59)	11-Jun(67.69)	12-Jun(59.66)	11-Jun(66.42)	11-Jun(54.45)
D25	June	1400	8-Jun	09-Jun(43.98)	07-Jun(46.12)	08-Jun(44.09)	07-Jun(49.08)	07-Jun(40.50)
D25	June	2000	8-Jun	10-Jun(37.52)	09-Jun(40.52)	09-Jun(38.01)	09-Jun(42.32)	08-Jun(34.33)
D25	June	2400	8-Jun	14-Jun(73.37)	14-Jun(72.71)	10-Jun(67.41)	10-Jun(75.05)	10-Jun(60.88)
D27	June	1400	5-Jun	09-Jun(40.62)	05-Jun(43.01)	05-Jun(40.24)	04-Jun(43.14)	04-Jun(37.50)
D27	June	2000	5-Jun	06-Jun(33.54)	05-Jun(35.73)	06-Jun(33.00)	05-Jun(36.74)	05-Jun(31.66)
D27	June	2400	5-Jun	11-Jun(29.50)	07-Jun(32.07)	07-Jun(29.57)	07-Jun(32.92)	07-Jun(27.16)
D27	June	3000	5-Jun	09-Jun(71.92)	08-Jun(74.37)	08-Jun(64.91)	08-Jun(75.76)	08-Jun(66.63)
D28	June	1400	4-Jun	04-Jun(20.24)	03-Jun(16.05)	03-Jun(18.59)	03-Jun(20.69)	02-Jun(18.34)
D28	June	2000	4-Jun	05-Jun(25.93)	04-Jun(25.23)	05-Jun(29.68)	04-Jun(28.12)	03-Jun(24.30)
D28	June	2400	4-Jun	06-Jun(42.67)	05-Jun(45.71)	06-Jun(43.24)	05-Jun(48.14)	05-Jun(40.00)
D30	June	1400	8-Jun	09-Jun(57.50)	08-Jun(61.49)	08-Jun(57.76)	08-Jun(64.31)	07-Jun(53.99)
D30	June	2000	8-Jun	10-Jun(39.56)	09-Jun(41.90)	09-Jun(39.20)	09-Jun(42.02)	08-Jun(36.53)
D30	June	2400	8-Jun	11-Jun(34.23)	10-Jun(36.46)	10-Jun(33.68)	10-Jun(37.50)	09-Jun(32.31)
D35	July	1400	2-Jul	01-Jul(47.64)	30-Jun(51.50)	01-Jul(51.23)	30-Jun(50.58)	30-Jun(45.78)
D35	July	2000	2-Jul	03-Jul(35.47)	03-Jul(34.32)	03-Jul(38.90)	03-Jul(36.64)	02-Jul(33.21)
D35	July	2400	2-Jul	05-Jul(30.13)	04-Jul(32.62)	04-Jul(32.28)	04-Jul(32.48)	03-Jul(28.48)
D39	July	1400	2-Jul	06-Jul(27.88)	05-Jul(30.31)	05-Jul(27.95)	05-Jul(31.11)	04-Jul(25.67)
D39	July	2000	15-Jul	18-Jul(52.69)	15-Jul(55.80)	15-Jul(52.20)	14-Jul(55.96)	14-Jul(48.64)
D39	July	2400	15-Jul	16-Jul(37.43)	15-Jul(40.46)	16-Jul(40.25)	15-Jul(39.74)	15-Jul(35.97)
D40	August	2000	15-Jul	17-Jul(29.43)	16-Jul(28.48)	17-Jul(32.28)	16-Jul(30.41)	16-Jul(27.56)
D43	August	1400	29-Jul	30-Jul(36.81)	30-Jul(34.34)	30-Jul(36.73)	30-Jul(38.74)	29-Jul(31.48)
D43	August	2000	12-Aug	13-Aug(49.71)	12-Aug(50.92)	12-Aug(46.38)	12-Aug(52.46)	11-Aug(46.50)
D43	August	2400	12-Aug	NA	16-Aug(49.89)	16-Aug(52.44)	13-Aug(47.26)	13-Aug(40.65)
D45	September	1400	28-Aug	01-Sep(29.83)	13-Aug(17.12)	14-Aug(18.31)	13-Aug(19.31)	13-Aug(15.69)
D45	September	2000	28-Aug	02-Sep(50.80)	29-Aug(51.92)	28-Aug(29.90)	28-Aug(33.28)	28-Aug(27.46)
D45	September	2400	28-Aug	31-Aug(36.03)	30-Aug(34.86)	29-Aug(55.28)	29-Aug(54.44)	28-Aug(48.77)
D51	September	2000	18-Sep	19-Sep(34.83)	30-Aug(39.52)	30-Aug(39.52)	30-Aug(37.23)	29-Aug(33.74)
D51	September	2400	18-Sep	21-Sep(28.78)	19-Sep(36.88)	19-Sep(34.50)	19-Sep(36.99)	18-Sep(32.15)
D53	October	1400	2-Oct	07-Oct(42.05)	19-Sep(31.29)	20-Sep(28.85)	19-Sep(32.12)	19-Sep(26.50)
D53	October	2400	2-Oct	10-Oct(66.77)	02-Oct(43.08)	02-Oct(39.23)	01-Oct(44.38)	01-Oct(37.46)
D54	October	1400	2-Oct	03-Oct(32.90)	05-Oct(61.98)	05-Oct(61.99)	05-Oct(69.01)	04-Oct(60.16)
D54	October	2000	2-Oct	04-Oct(58.97)	02-Oct(34.55)	02-Oct(33.26)	01-Oct(37.03)	01-Oct(30.23)
D54	October	2400	2-Oct	05-Oct(43.28)	02-Oct(63.06)	03-Oct(59.24)	02-Oct(65.95)	02-Oct(55.37)
D54	October	3000	2-Oct	11-Oct(40.22)	03-Oct(45.83)	04-Oct(42.88)	03-Oct(45.97)	02-Oct(39.96)
D61	November	1400	6-Nov	06-Nov(44.42)	07-Oct(43.72)	07-Oct(40.31)	06-Oct(44.88)	07-Oct(37.03)
D61	November	2000	6-Nov	10-Nov(27.85)	05-Nov(43.65)	05-Nov(50.85)	05-Nov(47.55)	04-Nov(42.14)
D61	November	2400	6-Nov	11-Nov(42.97)	10-Nov(45.50)	09-Nov(42.57)	07-Nov(45.64)	06-Nov(39.67)
D61	November	3000	6-Nov	15-Nov(41.88)	10-Nov(27.36)	11-Nov(31.88)	10-Nov(29.81)	09-Nov(26.42)
D61	November				12-Nov(44.88)	14-Nov(42.45)	12-Nov(47.26)	11-Nov(39.27)

Table B.13: Byrd and Butler TOP date mean estimation and percentage (%) ADH coverage by length in 2013. Grey cell indicates correct TOP prediction. F denotes fluctuating temperatures, C denotes constant temperatures.

BodyID	Month	Milestone	Actual TOP	F 15.6° C min	F 15.6° C max	F 21.1° C min	F 21.1° C max	C 25° C min	C 25° C max	F 26.7° C min	F 26.7° C max	F 32.2° C min	F 32.2° C max
D51	September	1400	22-Sep	19-Sep(78.85)	21-Sep(50.63)	19-Sep(95.73)	21-Sep(50.75)	19-Sep(87.28)	21-Sep(45.98)	19-Sep(95.44)	21-Sep(51.19)	19-Sep(108.42)	21-Sep(41.52)
D51	September	2000	22-Sep	24-Sep(63.58)	26-Sep(42.23)	23-Sep(77.12)	26-Sep(39.40)	24-Sep(71.17)	26-Sep(39.40)	23-Sep(77.82)	26-Sep(44.57)	22-Sep(86.52)	26-Sep(39.50)
D51	September	2400	22-Sep	26-Sep(50.08)	28-Sep(27.25)	24-Sep(61.68)	27-Sep(29.20)	25-Sep(56.23)	27-Sep(27.62)	24-Sep(61.50)	26-Sep(30.75)	24-Sep(68.62)	28-Sep(25.55)
D53	October	1400	10-Oct	10-Oct(116.45)	11-Oct(74.77)	09-Oct(141.39)	11-Oct(74.96)	10-Oct(128.92)	12-Oct(67.91)	09-Oct(140.96)	11-Oct(75.61)	09-Oct(160.13)	12-Oct(61.33)
D53	October	2000	10-Oct	NA	NA	11-Oct(78.29)	12-Oct(68.94)	11-Oct(82.11)	12-Oct(75.01)	11-Oct(87.90)	12-Oct(65.05)	11-Oct(84.13)	13-Oct(54.92)
D53	October	2400	10-Oct	12-Oct(64.93)	14-Oct(37.00)	11-Oct(79.96)	14-Oct(38.80)	11-Oct(72.25)	14-Oct(37.08)	11-Oct(79.01)	14-Oct(41.29)	10-Oct(88.95)	14-Oct(34.07)
D53	October	3000	10-Oct	15-Oct(100.92)	19-Oct(70.87)	14-Oct(125.97)	19-Oct(73.29)	14-Oct(115.55)	20-Oct(63.97)	14-Oct(126.35)	18-Oct(74.66)	13-Oct(140.48)	20-Oct(65.66)
D54	October	2000	11-Oct	12-Oct(71.08)	14-Oct(52.81)	11-Oct(93.37)	13-Oct(85.64)	12-Oct(85.64)	14-Oct(50.47)	11-Oct(93.65)	13-Oct(56.19)	11-Oct(104.12)	14-Oct(48.66)
D54	October	2400	11-Oct	14-Oct(60.70)	15-Oct(45.10)	12-Oct(79.73)	15-Oct(47.36)	13-Oct(73.14)	15-Oct(43.10)	12-Oct(79.97)	15-Oct(47.98)	12-Oct(88.91)	16-Oct(41.56)
D54	October	3000	11-Oct	17-Oct(50.14)	21-Oct(32.19)	15-Oct(60.88)	21-Oct(32.27)	15-Oct(55.51)	22-Oct(29.24)	15-Oct(60.70)	21-Oct(32.56)	14-Oct(68.95)	23-Oct(26.41)

Table B.14: Byrd and Butler TOP date mean estimation and percentage (%) ADH coverage by length in 2016. Grey cell indicates correct TOP prediction. F denotes fluctuating temperatures, C denotes constant temperatures.

BodyID	Month	Milestone	Actual TOP	F 15.6° C min	F 15.6° C max	F 21.1° C min	F 21.1° C max	C 25° C min	C 25° C max	F 26.7° C min	F 26.7° C max	F 32.2° C min	F 32.2° C max
D06	March	2000	19-Feb	22-Feb(52.89)	29-Feb(28.48)	20-Feb(64.90)	28-Feb(30.34)	21-Feb(59.16)	29-Feb(28.03)	20-Feb(64.71)	28-Feb(31.20)	19-Feb(72.20)	29-Feb(26.88)
D06	March	2400	19-Feb	25-Feb(113.42)	01-Mar(65.29)	22-Feb(138.54)	01-Mar(70.51)	23-Feb(126.68)	01-Mar(66.14)	22-Feb(138.54)	29-Feb(73.64)	21-Feb(155.96)	01-Mar(59.73)
D08	March	1400	29-Feb	26-Feb(116.83)	02-Mar(63.57)	24-Feb(143.88)	01-Mar(68.11)	25-Feb(131.15)	01-Mar(64.43)	24-Feb(143.45)	01-Mar(71.73)	22-Feb(160.05)	02-Mar(59.59)
D08	March	2000	29-Feb	28-Feb(104.34)	04-Mar(60.06)	26-Feb(127.45)	03-Mar(64.87)	27-Feb(116.54)	04-Mar(60.85)	26-Feb(127.45)	03-Mar(67.74)	24-Feb(143.47)	04-Mar(54.95)
D08	March	2400	29-Feb	03-Mar(72.86)	06-Mar(50.61)	01-Mar(89.84)	06-Mar(51.26)	02-Mar(63.42)	07-Mar(46.18)	01-Mar(91.22)	06-Mar(52.24)	28-Feb(101.42)	07-Mar(44.10)
D08	March	3000	29-Feb	06-Mar(62.94)	09-Mar(42.12)	04-Mar(77.38)	09-Mar(43.15)	05-Mar(70.98)	10-Mar(39.29)	04-Mar(77.62)	09-Mar(44.45)	02-Mar(86.29)	10-Mar(37.52)
D23	June	1400	16-Jun	16-Jun(73.81)	17-Jun(55.14)	15-Jun(96.36)	17-Jun(57.58)	16-Jun(88.93)	17-Jun(52.41)	15-Jun(97.24)	17-Jun(58.34)	15-Jun(110.46)	17-Jun(51.71)
D23	June	2000	16-Jun	17-Jun(64.48)	18-Jun(37.72)	17-Jun(72.01)	18-Jun(40.09)	17-Jun(56.05)	18-Jun(37.60)	16-Jun(78.76)	18-Jun(41.86)	16-Jun(88.66)	19-Jun(33.96)
D23	June	2400	16-Jun	18-Jun(49.93)	19-Jun(27.05)	17-Jun(61.49)	19-Jun(29.11)	19-Jun(122.49)	21-Jun(44.53)	19-Jun(133.94)	21-Jun(71.84)	18-Jun(152.15)	21-Jun(58.27)
D23	June	3000	16-Jun	20-Jun(110.65)	21-Jun(71.05)	19-Jun(134.35)	21-Jun(71.22)	20-Jun(91.41)	22-Jun(44.90)	20-Jun(99.99)	22-Jun(49.99)	20-Jun(111.55)	23-Jun(41.54)
D26	June	1400	21-Jun	21-Jun(81.42)	22-Jun(44.30)	20-Jun(100.28)	22-Jun(47.47)	24-Jun(55.04)	24-Jun(35.04)	24-Jun(34.51)	24-Jun(34.51)	24-Jun(30.91)	24-Jun(25.93)
D26	June	2000	21-Jun	24-Jun(32.20)	25-Jun(31.26)	23-Jun(60.15)	25-Jun(30.98)	23-Jun(55.00)	25-Jun(28.72)	23-Jun(60.70)	25-Jun(31.97)	22-Jun(65.56)	25-Jun(25.93)
D26	June	2400	21-Jun	23-Jun(49.25)	26-Jun(67.53)	24-Jun(143.30)	26-Jun(72.94)	24-Jun(131.03)	26-Jun(68.41)	24-Jun(143.30)	26-Jun(76.17)	23-Jun(161.32)	26-Jun(61.78)
D28	June	3000	21-Jun	25-Jun(117.32)	30-Jun(62.19)	24-Jun(90.65)	26-Jun(50.54)	14-Jun(36.27)	15-Jun(46.03)	13-Jun(90.92)	15-Jun(40.38)	13-Jun(101.09)	15-Jun(43.95)
D30	June	1400	30-Jun	14-Jun(73.73)	15-Jun(49.34)	29-Jun(75.97)	15-Jun(50.54)	29-Jun(63.15)	01-Jul(36.27)	29-Jun(76.65)	01-Jul(40.38)	29-Jun(82.79)	01-Jul(32.75)
D67	February	1400	26-Jan	30-Jun(62.19)	01-Jul(39.47)	21-Jan(90.78)	31-Jan(54.25)	01-Jul(49.37)	01-Feb(55.54)	30-Jan(71.67)	02-Feb(50.59)	15-Jan(104.06)	01-Feb(48.71)
D67	February	2000	26-Jan	29-Jan(69.55)	NA	01-Feb(62.12)	02-Feb(51.38)	31-Jan(65.64)	02-Feb(55.54)	30-Jan(71.67)	02-Feb(50.59)	31-Jan(69.12)	06-Feb(43.90)
D67	February	2400	26-Jan	NA	NA	09-Feb(94.87)	09-Feb(94.87)	02-Feb(114.08)	06-Feb(106.47)	02-Feb(118.54)	09-Feb(91.93)	02-Feb(115.77)	10-Feb(83.61)

Table B.15: Byrd and Butler TOP date mean estimation and percentage (%) ADH coverage by length in 2015. Grey cell indicates correct TOP prediction. F denotes fluctuating temperatures, C denotes constant temperatures.

BodyID	Month	Milestone	Actual TOP	F 15.6°C min	F 15.6°C max	F 21.1°C min	F 21.1°C max	C 25°C min	C 25°C max	F 26.7°C min	F 26.7°C max	F 32.2°C min	F 32.2°C max
D23	June	1400	8-Jun	09-Jun(100.57)	10-Jun(63.83)	08-Jun(122.85)	10-Jun(63.26)	08-Jun(112.33)	10-Jun(58.05)	08-Jun(123.95)	10-Jun(65.30)	07-Jun(133.88)	10-Jun(52.96)
D23	June	2000	8-Jun	10-Jun(76.72)	11-Jun(42.88)	09-Jun(94.49)	11-Jun(45.85)	09-Jun(86.13)	10-Jun(43.07)	09-Jun(94.21)	11-Jun(47.95)	08-Jun(105.11)	11-Jun(40.26)
D23	June	2400	8-Jun	11-Jun(63.47)	12-Jun(40.75)	10-Jun(77.06)	12-Jun(42.71)	10-Jun(70.26)	12-Jun(37.64)	09-Jun(94.21)	12-Jun(41.91)	09-Jun(85.42)	12-Jun(34.35)
D23	June	3000	8-Jun	12-Jun(51.11)	13-Jun(33.32)	11-Jun(62.55)	13-Jun(34.52)	10-Jun(76.19)	14-Jun(30.93)	11-Jun(62.10)	13-Jun(34.44)	11-Jun(69.04)	14-Jun(30.02)
D24	June	1400	8-Jun	08-Jun(102.55)	10-Jun(63.52)	08-Jun(114.12)	10-Jun(63.52)	08-Jun(114.12)	10-Jun(58.57)	07-Jun(124.80)	11-Jun(40.50)	10-Jun(140.50)	10-Jun(53.81)
D24	June	2000	8-Jun	09-Jun(81.42)	11-Jun(45.20)	09-Jun(100.27)	11-Jun(47.47)	09-Jun(100.27)	11-Jun(45.70)	09-Jun(99.98)	11-Jun(50.88)	08-Jun(111.54)	11-Jun(41.53)
D24	June	2400	8-Jun	10-Jun(64.34)	12-Jun(41.31)	10-Jun(78.11)	12-Jun(41.41)	10-Jun(71.22)	12-Jun(37.52)	10-Jun(77.88)	12-Jun(41.77)	09-Jun(88.47)	12-Jun(33.88)
D24	June	3000	8-Jun	12-Jun(97.25)	13-Jun(67.56)	11-Jun(119.93)	13-Jun(68.43)	12-Jun(111.36)	10-Jun(61.64)	11-Jun(121.76)	13-Jun(69.74)	11-Jun(135.38)	14-Jun(58.86)
D25	June	1400	8-Jun	08-Jun(76.62)	10-Jun(49.94)	07-Jun(93.93)	10-Jun(51.75)	08-Jun(85.13)	10-Jun(46.37)	07-Jun(93.09)	10-Jun(51.62)	07-Jun(103.49)	10-Jun(58.00)
D25	June	2000	8-Jun	09-Jun(63.98)	11-Jun(42.81)	09-Jun(78.67)	11-Jun(43.86)	09-Jun(72.16)	11-Jun(39.94)	09-Jun(78.90)	10-Jun(45.19)	08-Jun(87.72)	11-Jun(38.14)
D25	June	2400	8-Jun	11-Jun(103.23)	12-Jun(78.49)	10-Jun(140.35)	11-Jun(62.86)	10-Jun(127.97)	12-Jun(75.41)	10-Jun(139.93)	11-Jun(83.96)	09-Jun(135.57)	12-Jun(74.40)
D27	June	1400	5-Jun	05-Jun(75.40)	06-Jun(48.41)	04-Jun(91.54)	06-Jun(50.73)	05-Jun(83.46)	07-Jun(44.71)	04-Jun(91.26)	06-Jun(49.78)	04-Jun(101.47)	07-Jun(40.81)
D27	June	2000	5-Jun	06-Jun(61.84)	08-Jun(35.60)	05-Jun(75.53)	08-Jun(38.44)	06-Jun(69.06)	08-Jun(36.06)	05-Jun(75.53)	08-Jun(40.15)	05-Jun(85.03)	08-Jun(32.56)
D27	June	2400	5-Jun	07-Jun(49.87)	09-Jun(35.02)	06-Jun(62.25)	08-Jun(36.22)	07-Jun(57.10)	09-Jun(31.61)	06-Jun(62.44)	08-Jun(36.90)	06-Jun(69.42)	09-Jun(32.45)
D27	June	3000	5-Jun	09-Jun(97.32)	10-Jun(72.70)	08-Jun(127.05)	10-Jun(75.92)	08-Jun(117.25)	10-Jun(69.10)	08-Jun(128.21)	10-Jun(76.93)	07-Jun(145.65)	10-Jun(68.17)
D28	June	1400	4-Jun	06-Jun(33.83)	06-Jun(33.83)	06-Jun(33.24)	06-Jun(33.24)	06-Jun(38.72)	06-Jun(38.72)	06-Jun(36.21)	06-Jun(36.21)	06-Jun(32.09)	06-Jun(32.09)
D28	June	2000	4-Jun	07-Jun(33.00)	07-Jun(33.00)	04-Jun(78.50)	07-Jun(34.58)	04-Jun(73.25)	07-Jun(33.47)	07-Jun(33.04)	07-Jun(33.04)	07-Jun(30.84)	07-Jun(30.84)
D28	June	2400	4-Jun	06-Jun(78.42)	08-Jun(44.69)	05-Jun(96.00)	08-Jun(48.57)	06-Jun(87.26)	08-Jun(44.79)	05-Jun(95.43)	08-Jun(49.86)	05-Jun(107.43)	08-Jun(41.14)
D30	June	1400	8-Jun	08-Jun(102.52)	10-Jun(65.82)	08-Jun(124.48)	10-Jun(68.99)	08-Jun(113.49)	10-Jun(60.80)	08-Jun(124.10)	10-Jun(67.69)	07-Jun(137.97)	10-Jun(55.49)
D30	June	2000	8-Jun	10-Jun(73.44)	11-Jun(46.61)	09-Jun(89.70)	11-Jun(46.19)	09-Jun(82.02)	11-Jun(42.83)	09-Jun(90.51)	11-Jun(47.68)	08-Jun(97.76)	11-Jun(38.67)
D30	June	2400	8-Jun	11-Jun(63.10)	12-Jun(40.52)	10-Jun(76.62)	12-Jun(42.46)	10-Jun(69.85)	12-Jun(37.42)	10-Jun(76.38)	12-Jun(41.66)	09-Jun(84.92)	12-Jun(34.15)
D35	July	1400	2-Jul	01-Jul(98.52)	03-Jul(53.05)	30-Jun(120.89)	03-Jul(56.51)	01-Jul(110.20)	03-Jul(52.20)	30-Jun(120.54)	03-Jul(58.12)	30-Jun(134.48)	03-Jul(50.07)
D35	July	2000	2-Jul	03-Jul(75.96)	05-Jul(41.33)	03-Jul(95.54)	05-Jul(44.28)	03-Jul(85.27)	05-Jul(41.89)	03-Jul(93.27)	05-Jul(46.64)	02-Jul(104.06)	05-Jul(38.75)
D35	July	2400	2-Jul	04-Jul(63.27)	06-Jul(34.07)	04-Jul(77.63)	06-Jul(36.29)	04-Jul(70.77)	06-Jul(33.52)	04-Jul(77.41)	06-Jul(37.52)	03-Jul(86.36)	06-Jul(32.16)
D39	July	1400	2-Jul	06-Jul(48.93)	07-Jul(27.88)	05-Jul(59.90)	07-Jul(30.31)	05-Jul(54.45)	07-Jul(27.95)	05-Jul(59.55)	07-Jul(31.11)	04-Jul(67.03)	07-Jul(25.67)
D39	July	2000	15-Jul	15-Jul(97.80)	16-Jul(62.80)	14-Jul(118.75)	16-Jul(65.81)	15-Jul(108.27)	16-Jul(58.00)	14-Jul(118.39)	16-Jul(64.57)	14-Jul(131.62)	17-Jul(52.94)
D39	July	2400	15-Jul	17-Jul(63.27)	18-Jul(34.07)	16-Jul(77.63)	18-Jul(36.29)	17-Jul(70.77)	19-Jul(33.52)	16-Jul(77.41)	18-Jul(37.32)	16-Jul(86.36)	19-Jul(32.16)
D40	August	1400	29-Jul	01-Aug(50.53)	01-Aug(50.53)	30-Jul(120.18)	01-Aug(52.94)	30-Jul(112.14)	01-Aug(51.23)	01-Aug(50.58)	01-Aug(50.58)	01-Aug(47.21)	01-Aug(47.21)
D43	August	1400	12-Aug	12-Aug(69.53)	13-Aug(51.66)	12-Aug(91.33)	13-Aug(54.24)	12-Aug(83.78)	14-Aug(49.37)	12-Aug(91.60)	13-Aug(54.96)	11-Aug(101.85)	13-Aug(47.60)
D43	August	2000	12-Aug	NA	NA	13-Aug(61.44)	14-Aug(50.81)	13-Aug(64.92)	14-Aug(25.06)	13-Aug(70.89)	14-Aug(50.04)	13-Aug(68.37)	14-Aug(43.42)
D43	August	2400	12-Aug	16-Aug(23.39)	16-Aug(23.39)	15-Aug(25.32)	15-Aug(25.32)	15-Aug(25.06)	15-Aug(25.06)	15-Aug(25.21)	15-Aug(25.21)	16-Aug(22.11)	16-Aug(22.11)
D45	September	1400	28-Aug	29-Aug(47.92)	30-Aug(35.79)	28-Aug(62.56)	29-Aug(37.38)	28-Aug(57.73)	30-Aug(34.02)	28-Aug(63.13)	29-Aug(37.88)	27-Aug(71.71)	30-Aug(33.57)
D45	September	2000	28-Aug	30-Aug(107.55)	31-Aug(61.91)	29-Aug(131.36)	31-Aug(66.86)	29-Aug(120.12)	31-Aug(62.72)	29-Aug(131.36)	31-Aug(69.82)	28-Aug(147.88)	31-Aug(56.64)
D45	September	2400	28-Aug	31-Aug(77.17)	30-Aug(95.04)	30-Aug(95.04)	01-Sep(46.11)	30-Aug(85.87)	01-Sep(44.08)	30-Aug(93.91)	01-Sep(49.07)	29-Aug(105.72)	01-Sep(40.49)
D51	September	2000	18-Sep	19-Sep(64.88)	21-Sep(36.97)	18-Sep(79.44)	21-Sep(40.19)	19-Sep(72.20)	21-Sep(37.06)	19-Sep(78.96)	21-Sep(41.26)	18-Sep(88.89)	21-Sep(34.04)
D51	September	2400	18-Sep	20-Sep(49.40)	22-Sep(33.06)	19-Sep(60.74)	21-Sep(33.86)	20-Sep(55.71)	22-Sep(30.84)	19-Sep(60.92)	21-Sep(34.89)	19-Sep(67.73)	22-Sep(29.45)
D53	October	1400	2-Oct	02-Oct(58.82)	04-Oct(43.94)	01-Oct(76.79)	03-Oct(45.89)	02-Oct(70.87)	04-Oct(41.76)	03-Oct(46.49)	03-Oct(46.49)	01-Oct(88.03)	04-Oct(41.20)
D53	October	2000	2-Oct	06-Oct(94.46)	07-Oct(70.18)	05-Oct(124.08)	07-Oct(73.69)	05-Oct(113.81)	07-Oct(67.07)	05-Oct(124.45)	07-Oct(74.67)	04-Oct(138.36)	07-Oct(64.67)
D54	October	1400	2-Oct	02-Oct(57.95)	04-Oct(38.78)	01-Oct(71.25)	04-Oct(39.73)	02-Oct(65.36)	04-Oct(36.18)	01-Oct(71.46)	04-Oct(40.93)	01-Oct(79.46)	04-Oct(34.55)
D54	October	2000	2-Oct	04-Oct(103.20)	05-Oct(69.06)	02-Oct(126.89)	05-Oct(70.75)	03-Oct(116.40)	06-Oct(64.43)	02-Oct(127.27)	05-Oct(72.89)	02-Oct(141.50)	06-Oct(61.52)
D54	October	2400	2-Oct	04-Oct(80.63)	07-Oct(45.95)	03-Oct(98.72)	06-Oct(49.95)	04-Oct(77.84)	07-Oct(45.87)	03-Oct(98.13)	06-Oct(51.28)	02-Oct(110.47)	07-Oct(42.31)
D54	October	3000	2-Oct	07-Oct(64.61)	08-Oct(48.00)	06-Oct(84.86)	08-Oct(50.40)	06-Oct(77.84)	09-Oct(45.87)	06-Oct(85.12)	08-Oct(51.07)	05-Oct(94.63)	09-Oct(44.23)
D61	November	1400	6-Nov	06-Nov(103.65)	10-Nov(55.43)	05-Nov(127.18)	10-Nov(58.70)	05-Nov(115.93)	10-Nov(46.51)	05-Nov(127.94)	10-Nov(58.88)	10-Nov(51.17)	10-Nov(42.00)
D61	November	2000	6-Nov	10-Nov(79.76)	12-Nov(45.91)	07-Nov(97.42)	10-Nov(49.59)	08-Nov(89.08)	12-Nov(46.51)	07-Nov(97.42)	12-Nov(51.78)	06-Nov(109.67)	13-Nov(42.00)
D61	November	2400	6-Nov	11-Nov(64.74)	15-Nov(35.94)	10-Nov(79.73)	15-Nov(37.74)	10-Nov(72.68)	15-Nov(36.34)	10-Nov(79.49)	14-Nov(40.46)	08-Nov(88.69)	16-Nov(33.02)
D61	November	3000	6-Nov	15-Nov(76.98)	19-Nov(41.88)	12-Nov(94.80)	19-Nov(44.88)	14-Nov(86.42)	19-Nov(42.45)	12-Nov(94.52)	18-Nov(47.26)	11-Nov(105.46)	19-Nov(39.27)

Table B.16: Byrd and Butler TOP date maximum estimation and percentage (%) ADH coverage by length in 2013. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Fluctuating 15.6° C	Fluctuating 21.1° C	Constant 25° C	Fluctuating 26.7° C	Fluctuating 32.2° C
D51	September	1400	22-Sep	21-Sep(72.45)	21-Sep(94.58)	21-Sep(87.28)	21-Sep(95.44)	21-Sep(108.42)
D51	September	2000	22-Sep	26-Sep(55.99)	26-Sep(77.12)	26-Sep(71.17)	26-Sep(76.40)	26-Sep(84.64)
D51	September	2400	22-Sep	28-Sep(49.90)	28-Sep(60.95)	28-Sep(55.73)	28-Sep(60.95)	28-Sep(68.62)
D53	October	1400	10-Oct	NA	12-Oct(126.06)	12-Oct(122.01)	12-Oct(137.12)	12-Oct(149.91)
D53	October	2000	10-Oct	NA	NA	NA	NA	13-Oct(79.46)
D53	October	2400	10-Oct	14-Oct(63.49)	14-Oct(78.07)	14-Oct(71.61)	14-Oct(78.30)	15-Oct(87.06)
D53	October	3000	10-Oct	20-Oct(90.90)	19-Oct(125.21)	20-Oct(115.55)	19-Oct(124.06)	21-Oct(137.43)
D54	October	2000	11-Oct	NA	14-Oct(78.65)	14-Oct(79.53)	14-Oct(88.54)	15-Oct(86.01)
D54	October	2400	11-Oct	16-Oct(57.54)	15-Oct(79.25)	17-Oct(73.14)	15-Oct(78.52)	17-Oct(86.98)
D54	October	3000	11-Oct	21-Oct(50.14)	21-Oct(60.88)	22-Oct(55.51)	21-Oct(60.70)	23-Oct(68.95)

Table B.17: Byrd and Butler TOP date maximum estimation and percentage (%) ADH coverage by length in 2016. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Fluctuating 15.6° C	Fluctuating 21.1° C	Constant 25° C	Fluctuating 26.7° C	Fluctuating 32.2° C
D06	March	2000	19-Feb	29-Feb(52.89)	28-Feb(64.90)	29-Feb(59.16)	28-Feb(64.71)	29-Feb(72.20)
D06	March	2400	19-Feb	01-Mar(113.42)	01-Mar(138.54)	01-Mar(126.68)	29-Feb(138.54)	01-Mar(155.96)
D08	March	1400	29-Feb	02-Mar(116.40)	02-Mar(142.17)	02-Mar(130.00)	02-Mar(142.17)	03-Mar(160.05)
D08	March	2000	29-Feb	04-Mar(93.17)	04-Mar(126.68)	04-Mar(115.51)	04-Mar(126.30)	05-Mar(140.42)
D08	March	2400	29-Feb	NA	07-Mar(81.58)	07-Mar(78.95)	07-Mar(88.73)	08-Mar(97.01)
D23	June	1400	16-Jun	10-Mar(55.84)	10-Mar(76.91)	10-Mar(70.98)	09-Mar(76.21)	10-Mar(84.42)
D23	June	2000	16-Jun	NA	17-Jun(86.96)	17-Jun(84.17)	17-Jun(94.59)	17-Jun(103.41)
D23	June	2400	16-Jun	19-Jun(59.24)	19-Jun(77.34)	19-Jun(71.38)	19-Jun(78.05)	19-Jun(88.66)
D23	June	3000	16-Jun	20-Jun(49.19)	20-Jun(59.67)	19-Jun(55.07)	19-Jun(60.21)	20-Jun(66.95)
D26	June	1400	21-Jun	21-Jun(110.65)	21-Jun(134.35)	21-Jun(122.49)	21-Jun(133.94)	21-Jun(152.15)
D26	June	2000	21-Jun	23-Jun(81.13)	23-Jun(98.50)	23-Jun(89.81)	23-Jun(98.20)	23-Jun(109.18)
D26	June	2400	21-Jun	25-Jun(68.42)	25-Jun(84.26)	25-Jun(76.81)	25-Jun(84.01)	25-Jun(93.73)
D26	June	3000	21-Jun	25-Jun(45.25)	25-Jun(59.43)	25-Jun(54.52)	25-Jun(59.61)	25-Jun(66.28)
D28	June	1400	21-Jun	15-Jun(113.42)	26-Jun(141.58)	26-Jun(129.87)	26-Jun(142.01)	26-Jun(157.89)
D30	June	2000	21-Jun	15-Jun(73.73)	15-Jun(90.65)	15-Jun(83.15)	15-Jun(90.92)	15-Jun(101.09)
D30	June	2400	30-Jun	01-Jul(60.13)	01-Jul(75.06)	01-Jul(68.85)	01-Jul(75.28)	02-Jul(83.70)
D67	February	1400	26-Jan	NA	31-Jan(84.14)	01-Feb(80.04)	31-Jan(89.11)	01-Feb(98.53)
D67	February	2000	26-Jan	NA	NA	02-Feb(65.64)	04-Feb(66.05)	08-Feb(67.26)
D67	February	2400	26-Jan	NA	NA	NA	NA	11-Feb(109.34)

Table B.18: Byrd and Butler TOP date maximum estimation and percentage (%) ADH coverage by length in 2015. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Fluctuating 15.6° C	Fluctuating 21.1° C	Constant 25° C	Fluctuating 26.7° C	Fluctuating 32.2° C
D23	June	1400	8-Jun	10-Jun(92.41)	10-Jun(120.64)	10-Jun(111.33)	10-Jun(121.74)	10-Jun(138.29)
D23	June	2000	8-Jun	12-Jun(75.60)	11-Jun(91.69)	11-Jun(84.62)	11-Jun(92.53)	12-Jun(102.88)
D23	June	2400	8-Jun	12-Jun(55.74)	12-Jun(76.13)	12-Jun(70.26)	12-Jun(76.83)	12-Jun(85.42)
D24	June	3000	8-Jun	14-Jun(49.60)	14-Jun(61.16)	14-Jun(56.79)	13-Jun(62.10)	14-Jun(69.04)
D24	June	1400	8-Jun	10-Jun(101.04)	10-Jun(122.56)	10-Jun(113.11)	10-Jun(123.68)	10-Jun(137.51)
D24	June	2000	8-Jun	11-Jun(81.12)	11-Jun(99.08)	11-Jun(90.60)	11-Jun(99.98)	11-Jun(107.98)
D24	June	2400	8-Jun	12-Jun(63.62)	12-Jun(77.17)	12-Jun(71.22)	12-Jun(77.88)	12-Jun(86.58)
D24	June	3000	8-Jun	13-Jun(92.43)	13-Jun(120.66)	14-Jun(111.36)	13-Jun(121.76)	14-Jun(138.32)
D25	June	1400	8-Jun	10-Jun(70.66)	10-Jun(92.24)	10-Jun(85.13)	10-Jun(93.09)	10-Jun(105.74)
D25	June	2000	8-Jun	11-Jun(59.89)	11-Jun(78.67)	11-Jun(72.16)	11-Jun(78.90)	11-Jun(87.72)
D25	June	2400	8-Jun	NA	NA	12-Jun(116.54)	12-Jun(122.12)	12-Jun(118.37)
D27	June	1400	5-Jun	08-Jun(59.78)	07-Jun(81.62)	07-Jun(78.99)	07-Jun(88.77)	07-Jun(97.06)
D27	June	2000	5-Jun	NA	08-Jun(73.72)	08-Jun(68.45)	08-Jun(74.85)	08-Jun(83.22)
D27	June	2400	5-Jun	NA	09-Jun(52.44)	09-Jun(53.02)	09-Jun(59.03)	09-Jun(57.35)
D27	June	3000	5-Jun	10-Jun(92.24)	10-Jun(127.05)	10-Jun(117.25)	10-Jun(125.88)	10-Jun(139.45)
D28	June	1400	4-Jun	07-Jun(78.64)	07-Jun(96.85)	07-Jun(88.29)	07-Jun(96.57)	07-Jun(107.74)
D28	June	2000	4-Jun	07-Jun(64.12)	07-Jun(78.97)	07-Jun(71.35)	07-Jun(78.03)	07-Jun(87.85)
D28	June	2400	4-Jun	08-Jun(78.13)	08-Jun(94.86)	08-Jun(86.49)	08-Jun(94.57)	08-Jun(105.14)
D30	June	1400	8-Jun	10-Jun(94.20)	10-Jun(123.73)	10-Jun(113.49)	10-Jun(124.10)	10-Jun(137.97)
D30	June	2000	8-Jun	11-Jun(67.48)	11-Jun(88.09)	11-Jun(81.30)	11-Jun(88.89)	12-Jun(100.98)
D30	June	2400	8-Jun	12-Jun(55.42)	12-Jun(75.69)	12-Jun(69.85)	12-Jun(76.38)	12-Jun(84.92)
D35	July	1400	2-Jul	03-Jul(98.16)	03-Jul(120.18)	03-Jul(109.23)	03-Jul(119.46)	03-Jul(134.48)
D35	July	2000	2-Jul	05-Jul(75.68)	05-Jul(91.88)	05-Jul(83.78)	05-Jul(91.60)	06-Jul(104.06)
D35	July	2400	2-Jul	06-Jul(63.04)	06-Jul(77.17)	06-Jul(70.15)	06-Jul(76.71)	06-Jul(86.36)
D35	July	3000	2-Jul	07-Jul(48.93)	07-Jul(59.90)	07-Jul(54.45)	07-Jul(59.55)	07-Jul(67.03)
D39	July	1400	15-Jul	NA	16-Jul(108.73)	17-Jul(103.43)	16-Jul(115.16)	17-Jul(127.33)
D39	July	2000	15-Jul	18-Jul(76.84)	18-Jul(93.29)	18-Jul(85.06)	18-Jul(93.01)	18-Jul(105.66)
D39	July	2400	15-Jul	19-Jul(62.80)	19-Jul(76.71)	19-Jul(70.15)	19-Jul(77.41)	19-Jul(83.60)
D40	August	2000	29-Jul	01-Aug(97.80)	01-Aug(119.46)	01-Aug(119.46)	01-Aug(119.46)	01-Aug(134.48)
D43	August	1400	12-Aug	13-Aug(66.46)	13-Aug(90.78)	13-Aug(83.78)	13-Aug(91.60)	13-Aug(101.85)
D43	August	2000	12-Aug	NA	NA	NA	14-Aug(62.55)	14-Aug(64.67)
D43	August	2400	12-Aug	16-Aug(48.93)	16-Aug(60.26)	16-Aug(54.93)	16-Aug(60.08)	16-Aug(67.03)
D45	September	1400	28-Aug	NA	NA	30-Aug(53.61)	30-Aug(53.94)	30-Aug(54.93)
D45	September	2000	28-Aug	NA	31-Aug(119.56)	31-Aug(113.74)	31-Aug(126.63)	31-Aug(140.02)
D45	September	2400	28-Aug	02-Sep(68.66)	02-Sep(93.35)	01-Sep(85.11)	01-Sep(93.06)	02-Sep(103.47)
D51	September	2000	18-Sep	21-Sep(63.93)	21-Sep(77.54)	21-Sep(71.56)	21-Sep(78.25)	21-Sep(87.00)
D51	September	2400	18-Sep	22-Sep(46.24)	22-Sep(60.37)	22-Sep(55.71)	22-Sep(60.92)	22-Sep(69.20)
D53	October	1400	2-Oct	NA	04-Oct(69.30)	04-Oct(67.07)	04-Oct(75.38)	04-Oct(82.41)
D53	October	2000	2-Oct	NA	07-Oct(114.30)	07-Oct(108.73)	07-Oct(121.05)	08-Oct(133.85)
D54	October	1400	2-Oct	04-Oct(51.41)	04-Oct(70.82)	04-Oct(65.36)	04-Oct(70.17)	05-Oct(77.73)
D54	October	2000	2-Oct	06-Oct(92.34)	06-Oct(126.12)	06-Oct(116.40)	06-Oct(127.27)	06-Oct(141.50)
D54	October	2400	2-Oct	07-Oct(77.67)	07-Oct(95.78)	07-Oct(88.94)	07-Oct(97.25)	07-Oct(108.12)
D54	October	3000	2-Oct	NA	09-Oct(68.92)	09-Oct(72.28)	09-Oct(77.38)	09-Oct(74.06)
D61	November	1400	6-Nov	11-Nov(102.89)	11-Nov(125.68)	10-Nov(114.92)	11-Nov(126.81)	11-Nov(136.96)
D61	November	2000	6-Nov	12-Nov(73.28)	12-Nov(96.25)	12-Nov(88.29)	12-Nov(96.54)	13-Nov(107.34)
D61	November	2400	6-Nov	16-Nov(62.36)	16-Nov(77.84)	16-Nov(71.40)	16-Nov(78.07)	16-Nov(86.80)
D61	November	3000	6-Nov	19-Nov(76.98)	19-Nov(94.80)	19-Nov(86.42)	18-Nov(94.52)	19-Nov(105.46)

APPENDIX C

THIRD APPENDIX - WEIGHT PHENOTYPE

C.1 Boatright and Tomberlin predictions

Table C.1: Boatright and Tomberlin TOP date minimum estimation and percentage (%) ADH coverage by weight in 2013. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D51	September	1400	22-Sep	21-Sep(59.67)	20-Sep(68.03)	21-Sep(54.47)
D51	September	2000	22-Sep	25-Sep(42.63)	25-Sep(51.94)	26-Sep(42.15)
D51	September	2400	22-Sep	27-Sep(23.02)	27-Sep(29.26)	27-Sep(23.27)
D53	October	1400	10-Oct	NA	NA	NA
D53	October	2000	10-Oct	19-Oct(57.26)	19-Oct(64.88)	10-Oct(51.96)
D53	October	2400	10-Oct	14-Oct(29.15)	13-Oct(34.24)	14-Oct(28.52)
D53	October	3000	10-Oct	18-Oct(27.48)	17-Oct(34.02)	19-Oct(27.66)
D54	October	2000	11-Oct	12-Oct(43.21)	12-Oct(53.49)	13-Oct(43.50)
D54	October	2400	11-Oct	15-Oct(36.60)	14-Oct(46.64)	15-Oct(38.55)
D54	October	3000	11-Oct	22-Oct(28.41)	21-Oct(36.21)	22-Oct(29.92)

Table C.2: Boatright and Tomberlin TOP date minimum estimation and percentage (%) ADH coverage by weight in 2016. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D06	March	2000	19-Feb	29-Feb(38.78)	28-Feb(44.21)	29-Feb(35.40)
D06	March	2400	19-Feb	01-Mar(33.63)	01-Mar(39.11)	01-Mar(32.16)
D08	March	1400	29-Feb	02-Mar(57.89)	01-Mar(67.32)	02-Mar(55.37)
D08	March	2000	29-Feb	04-Mar(28.58)	03-Mar(36.33)	05-Mar(28.90)
D08	March	2400	29-Feb	06-Mar(22.94)	05-Mar(29.16)	06-Mar(23.20)
D23	June	1400	29-Feb	08-Mar(27.26)	07-Mar(33.75)	09-Mar(27.44)
D23	June	2000	16-Jun	19-Jun(0.00)	19-Jun(0.00)	19-Jun(103.51)
D23	June	2400	16-Jun	18-Jun(47.34)	17-Jun(59.63)	18-Jun(49.62)
D23	June	3000	16-Jun	19-Jun(24.26)	18-Jun(30.83)	19-Jun(24.52)
D26	June	1400	16-Jun	21-Jun(25.95)	21-Jun(30.16)	21-Jun(24.81)
D26	June	2000	21-Jun	22-Jun(61.78)	21-Jun(71.85)	22-Jun(59.09)
D26	June	2400	21-Jun	NA	NA	NA
D26	June	3000	21-Jun	26-Jun(23.17)	25-Jun(27.21)	26-Jun(22.67)
D28	June	1400	21-Jun	16-Jun(63.76)	15-Jun(79.08)	15-Jun(65.29)
D30	June	1400	30-Jun	01-Jul(51.36)	01-Jul(60.32)	01-Jul(50.25)
D67	February	1400	26-Jan	02-Feb(37.13)	31-Jan(47.19)	01-Feb(37.54)
D67	February	2000	26-Jan	02-Feb(45.05)	01-Feb(57.52)	05-Feb(47.00)
D67	February	2400	26-Jan	18-Feb(56.58)	18-Feb(64.65)	01-Feb(49.98)

Table C.3: Boatright and Tomberlin TOP date minimum estimation and percentage (%) ADH coverage by weight in 2015. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D23	June	1400	8-Jun	09-Jun(50.11)	09-Jun(57.14)	10-Jun(45.74)
D23	June	2000	8-Jun	11-Jun(26.93)	11-Jun(34.23)	11-Jun(27.23)
D23	June	2400	8-Jun	11-Jun(33.26)	11-Jun(38.68)	12-Jun(31.81)
D23	June	3000	8-Jun	13-Jun(27.41)	13-Jun(33.99)	13-Jun(28.06)
D24	June	1400	8-Jun	10-Jun(51.50)	09-Jun(59.90)	10-Jun(49.26)
D24	June	2000	8-Jun	NA	NA	NA
D24	June	2400	8-Jun	12-Jun(33.52)	11-Jun(40.83)	12-Jun(33.14)
D24	June	3000	8-Jun	13-Jun(27.75)	13-Jun(35.37)	13-Jun(29.23)
D25	June	1400	8-Jun	09-Jun(54.98)	09-Jun(68.20)	10-Jun(56.30)
D25	June	2000	8-Jun	10-Jun(43.75)	10-Jun(55.75)	10-Jun(46.07)
D25	June	2400	8-Jun	14-Jun(55.76)	09-Jun(45.57)	11-Jun(37.66)
D27	June	1400	5-Jun	09-Jun(56.54)	09-Jun(70.13)	05-Jun(57.89)
D27	June	2000	5-Jun	07-Jun(39.17)	07-Jun(44.66)	08-Jun(35.75)
D27	June	2400	5-Jun	08-Jun(34.78)	08-Jun(43.06)	09-Jun(35.01)
D27	June	3000	5-Jun	09-Jun(30.67)	09-Jun(39.15)	10-Jun(31.99)
D28	June	1400	4-Jun	NA	NA	NA
D28	June	2000	4-Jun	NA	NA	NA
D28	June	2400	4-Jun	08-Jun(28.39)	07-Jun(33.34)	08-Jun(27.78)
D30	June	1400	8-Jun	10-Jun(52.97)	09-Jun(61.61)	10-Jun(50.67)
D30	June	2000	8-Jun	11-Jun(33.93)	10-Jun(39.85)	11-Jun(33.20)
D30	June	2400	8-Jun	11-Jun(23.19)	11-Jun(29.48)	12-Jun(23.45)
D35	July	1400	2-Jul	03-Jul(31.26)	03-Jul(41.39)	03-Jul(24.95)
D35	July	2000	2-Jul	05-Jul(26.83)	05-Jul(34.10)	05-Jul(27.13)
D35	July	2400	2-Jul	06-Jul(22.00)	06-Jul(27.97)	06-Jul(22.25)
D39	July	1400	15-Jul	08-Jul(26.43)	07-Jul(30.14)	08-Jul(24.13)
D39	July	2000	15-Jul	15-Jul(37.69)	15-Jul(47.91)	16-Jul(38.11)
D39	July	2400	15-Jul	17-Jul(27.88)	17-Jul(35.44)	17-Jul(28.19)
D40	August	2000	15-Jul	18-Jul(17.28)	18-Jul(22.88)	18-Jul(13.79)
D43	August	1400	29-Jul	01-Aug(27.80)	31-Jul(35.34)	01-Aug(28.11)
D43	August	2000	12-Aug	13-Aug(58.37)	13-Aug(74.38)	13-Aug(61.47)
D43	August	2400	12-Aug	16-Aug(54.35)	12-Aug(61.59)	14-Aug(49.32)
D45	September	1400	28-Aug	NA	NA	NA
D45	September	2000	28-Aug	29-Aug(54.92)	28-Aug(66.90)	29-Aug(54.29)
D45	September	2400	28-Aug	31-Aug(35.19)	30-Aug(41.33)	31-Aug(34.43)
D51	September	2000	18-Sep	NA	NA	NA
D51	September	2400	18-Sep	20-Sep(40.93)	20-Sep(49.86)	21-Sep(40.47)
D53	October	1400	2-Oct	21-Sep(34.17)	21-Sep(41.62)	22-Sep(33.78)
D53	October	2000	2-Oct	03-Oct(57.80)	03-Oct(71.69)	04-Oct(59.18)
D53	October	2400	2-Oct	07-Oct(33.19)	06-Oct(41.17)	07-Oct(33.99)
D54	October	1400	2-Oct	03-Oct(55.37)	03-Oct(64.40)	04-Oct(52.96)
D54	October	2000	2-Oct	05-Oct(42.88)	05-Oct(52.23)	05-Oct(42.39)
D54	October	2400	2-Oct	06-Oct(36.03)	06-Oct(41.08)	06-Oct(32.89)
D61	November	1400	6-Nov	06-Oct(29.32)	06-Oct(36.36)	08-Oct(30.02)
D61	November	2000	6-Nov	NA	NA	NA
D61	November	2400	6-Nov	NA	NA	NA
D61	November	3000	6-Nov	12-Nov(34.50)	11-Nov(42.03)	13-Nov(34.11)
D61	November			19-Nov(28.29)	17-Nov(35.09)	19-Nov(28.97)

Table C.4: Boatright and Tomberlin TOP date mean estimation and percentage (%) ADH coverage by weight in 2013. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C min	Pork/Equine 20.8° C max	Pork/Equine 24.3° C min	Pork/Equine 24.3° C max	Pork/Equine 28.2° C min	Pork/Equine 28.2° C max
D51	September	1400	22-Sep	21-Sep(62.16)	21-Sep(62.16)	21-Sep(75.72)	21-Sep(75.72)	21-Sep(61.45)	21-Sep(61.45)
D51	September	2000	22-Sep	21-Sep(46.61)	26-Sep(46.61)	25-Sep(58.71)	25-Sep(58.71)	26-Sep(48.86)	26-Sep(48.86)
D51	September	2400	22-Sep	28-Sep(34.07)	28-Sep(34.07)	27-Sep(39.62)	27-Sep(39.62)	28-Sep(32.58)	28-Sep(32.58)
D53	October	1400	10-Oct	12-Oct(58.69)	12-Oct(58.69)	11-Oct(72.79)	11-Oct(72.79)	12-Oct(60.09)	12-Oct(60.09)
D53	October	2000	10-Oct	11-Oct(85.89)	12-Oct(71.57)	11-Oct(87.48)	11-Oct(87.48)	12-Oct(64.02)	12-Oct(64.02)
D53	October	2400	10-Oct	14-Oct(35.26)	14-Oct(35.26)	14-Oct(42.96)	14-Oct(42.96)	14-Oct(34.86)	14-Oct(34.86)
D53	October	3000	10-Oct	20-Oct(29.26)	20-Oct(29.26)	18-Oct(36.85)	18-Oct(36.85)	20-Oct(30.67)	20-Oct(30.67)
D54	October	2000	11-Oct	14-Oct(56.11)	14-Oct(56.11)	13-Oct(62.41)	13-Oct(62.41)	14-Oct(51.06)	14-Oct(51.06)
D54	October	2400	11-Oct	15-Oct(45.75)	15-Oct(45.75)	15-Oct(50.89)	15-Oct(50.89)	16-Oct(41.63)	16-Oct(41.63)
D54	October	3000	11-Oct	22-Oct(28.41)	22-Oct(28.41)	21-Oct(36.21)	21-Oct(36.21)	22-Oct(29.92)	22-Oct(29.92)

Table C.5: Boatright and Tomberlin TOP date mean estimation and percentage (%) ADH coverage by weight in 2016. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C min	Pork/Equine 20.8° C max	Pork/Equine 24.3° C min	Pork/Equine 24.3° C max	Pork/Equine 28.2° C min	Pork/Equine 28.2° C max
D06	March	2000	19-Feb	29-Feb(38.78)	29-Feb(38.78)	28-Feb(44.21)	28-Feb(44.21)	29-Feb(35.40)	29-Feb(35.40)
D06	March	2400	19-Feb	01-Mar(33.63)	01-Mar(33.63)	01-Mar(39.11)	01-Mar(39.11)	01-Mar(32.16)	01-Mar(32.16)
D08	March	1400	29-Feb	02-Mar(56.32)	02-Mar(56.32)	01-Mar(64.22)	01-Mar(64.22)	03-Mar(51.41)	03-Mar(51.41)
D08	March	2000	29-Feb	04-Mar(44.02)	04-Mar(44.02)	03-Mar(54.50)	03-Mar(54.50)	04-Mar(44.32)	04-Mar(44.32)
D08	March	2400	29-Feb	06-Mar(44.51)	07-Mar(38.54)	05-Mar(49.21)	05-Mar(49.21)	07-Mar(40.21)	07-Mar(40.21)
D08	March	3000	29-Feb	10-Mar(29.03)	10-Mar(29.03)	09-Mar(36.56)	09-Mar(36.56)	10-Mar(30.43)	10-Mar(30.43)
D23	June	1400	16-Jun	17-Jun(81.89)	17-Jun(81.89)	16-Jun(92.79)	16-Jun(92.79)	17-Jun(74.31)	17-Jun(74.31)
D23	June	2000	16-Jun	19-Jun(44.45)	19-Jun(44.45)	18-Jun(55.04)	18-Jun(55.04)	19-Jun(44.75)	19-Jun(44.75)
D23	June	2400	16-Jun	19-Jun(36.38)	19-Jun(36.38)	19-Jun(44.32)	19-Jun(44.32)	19-Jun(35.97)	19-Jun(35.97)
D23	June	3000	16-Jun	21-Jun(25.93)	21-Jun(25.93)	21-Jun(30.16)	21-Jun(30.16)	21-Jun(24.81)	21-Jun(24.81)
D26	June	1400	21-Jun	23-Jun(60.11)	23-Jun(60.11)	22-Jun(68.54)	22-Jun(68.54)	23-Jun(54.87)	23-Jun(54.87)
D26	June	2000	21-Jun	25-Jun(33.14)	25-Jun(33.14)	24-Jun(38.93)	24-Jun(38.93)	25-Jun(32.43)	25-Jun(32.43)
D26	June	2400	21-Jun	25-Jun(34.08)	25-Jun(34.08)	25-Jun(42.19)	25-Jun(42.19)	25-Jun(34.31)	25-Jun(34.31)
D26	June	3000	21-Jun	26-Jun(28.77)	26-Jun(28.77)	26-Jun(35.62)	26-Jun(35.62)	26-Jun(28.97)	26-Jun(28.97)
D28	June	1400	21-Jun	16-Jun(63.76)	16-Jun(63.76)	15-Jun(79.08)	15-Jun(79.08)	15-Jun(65.29)	15-Jun(65.29)
D30	June	1400	30-Jun	01-Jul(61.30)	01-Jul(61.30)	01-Jul(71.29)	01-Jul(71.29)	02-Jul(58.63)	02-Jul(58.63)
D67	February	1400	26-Jan	31-Jan(77.22)	31-Jan(77.22)	NA	NA	31-Jan(70.07)	31-Jan(70.07)
D67	February	2000	26-Jan	NA	NA	NA	NA	01-Feb(70.49)	01-Feb(70.49)
D67	February	2400	26-Jan	NA	NA	NA	NA	01-Feb(79.20)	01-Feb(79.20)

Table C.6: Boatright and Tomberlin TOP date mean estimation and percentage (%) ADH coverage by weight in 2015. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C min	Pork/Equine 20.8° C max	Pork/Equine 24.3° C min	Pork/Equine 24.3° C max	Pork/Equine 28.2° C min	Pork/Equine 28.2° C max
D23	June	1400	8-Jun	10-Jun(57.07)	10-Jun(57.07)	10-Jun(71.88)	10-Jun(71.88)	10-Jun(59.82)	10-Jun(59.82)
D23	June	2000	8-Jun	11-Jun(40.39)	11-Jun(40.39)	11-Jun(49.20)	11-Jun(49.20)	11-Jun(39.93)	11-Jun(39.93)
D23	June	2400	8-Jun	12-Jun(35.96)	12-Jun(35.96)	12-Jun(45.82)	12-Jun(45.82)	12-Jun(37.87)	12-Jun(37.87)
D23	June	3000	8-Jun	14-Jun(28.45)	14-Jun(28.45)	13-Jun(35.83)	13-Jun(35.83)	14-Jun(29.82)	14-Jun(29.82)
D24	June	1400	8-Jun	10-Jun(52.20)	10-Jun(52.20)	10-Jun(63.59)	10-Jun(63.59)	10-Jun(51.61)	10-Jun(51.61)
D24	June	2000	8-Jun	11-Jun(40.39)	11-Jun(40.39)	11-Jun(49.20)	11-Jun(49.20)	11-Jun(39.93)	11-Jun(39.93)
D24	June	2400	8-Jun	12-Jun(35.31)	12-Jun(35.31)	11-Jun(43.79)	11-Jun(43.79)	12-Jun(36.15)	12-Jun(36.15)
D24	June	3000	8-Jun	13-Jun(34.69)	13-Jun(34.69)	13-Jun(38.59)	13-Jun(38.59)	13-Jun(31.57)	13-Jun(31.57)
D25	June	1400	8-Jun	09-Jun(67.51)	09-Jun(58.46)	09-Jun(74.65)	09-Jun(74.65)	10-Jun(60.99)	10-Jun(60.99)
D25	June	2000	8-Jun	10-Jun(54.68)	10-Jun(54.68)	10-Jun(60.82)	10-Jun(60.82)	11-Jun(49.76)	11-Jun(49.76)
D25	June	2400	8-Jun	11-Jun(47.82)	11-Jun(47.82)	11-Jun(54.45)	11-Jun(54.45)	12-Jun(43.69)	12-Jun(43.69)
D27	June	1400	5-Jun	06-Jun(76.58)	06-Jun(76.58)	06-Jun(87.19)	06-Jun(87.19)	06-Jun(69.96)	06-Jun(69.96)
D27	June	2000	5-Jun	08-Jun(43.52)	08-Jun(43.52)	07-Jun(55.46)	07-Jun(55.46)	08-Jun(45.84)	08-Jun(45.84)
D27	June	2400	5-Jun	08-Jun(43.81)	09-Jun(37.94)	08-Jun(48.44)	08-Jun(48.44)	09-Jun(39.58)	09-Jun(39.58)
D27	June	3000	5-Jun	10-Jun(37.97)	10-Jun(37.97)	09-Jun(43.02)	09-Jun(43.02)	10-Jun(34.45)	10-Jun(34.45)
D28	June	1400	4-Jun	07-Jun(27.63)	07-Jun(27.63)	07-Jun(36.58)	07-Jun(36.58)	07-Jun(22.06)	07-Jun(22.06)
D28	June	2000	4-Jun	08-Jun(28.86)	08-Jun(28.86)	07-Jun(36.69)	07-Jun(36.69)	08-Jun(29.18)	08-Jun(29.18)
D28	June	2400	4-Jun	08-Jun(34.34)	08-Jun(34.34)	08-Jun(41.83)	08-Jun(41.83)	08-Jun(33.95)	08-Jun(33.95)
D30	June	1400	8-Jun	10-Jun(56.55)	10-Jun(56.55)	10-Jun(70.14)	10-Jun(70.14)	10-Jun(57.91)	10-Jun(57.91)
D30	June	2000	8-Jun	11-Jun(43.23)	11-Jun(43.23)	11-Jun(53.62)	11-Jun(53.62)	11-Jun(44.27)	11-Jun(44.27)
D30	June	2400	8-Jun	12-Jun(37.11)	12-Jun(37.11)	12-Jun(47.29)	12-Jun(47.29)	12-Jun(39.09)	12-Jun(39.09)
D35	July	1400	2-Jul	03-Jul(51.00)	03-Jul(51.00)	03-Jul(59.91)	03-Jul(59.91)	03-Jul(49.91)	03-Jul(49.91)
D35	July	2000	2-Jul	05-Jul(38.63)	05-Jul(38.63)	05-Jul(44.05)	05-Jul(44.05)	05-Jul(35.26)	05-Jul(35.26)
D35	July	2400	2-Jul	06-Jul(31.68)	06-Jul(31.68)	06-Jul(36.12)	06-Jul(36.12)	06-Jul(28.92)	06-Jul(28.92)
D39	July	1400	15-Jul	16-Jul(61.81)	16-Jul(61.81)	16-Jul(77.85)	16-Jul(77.85)	16-Jul(64.78)	16-Jul(64.78)
D39	July	2000	15-Jul	18-Jul(41.82)	18-Jul(41.82)	17-Jul(50.94)	17-Jul(50.94)	18-Jul(41.35)	18-Jul(41.35)
D39	July	2400	15-Jul	19-Jul(32.73)	19-Jul(32.73)	18-Jul(37.32)	18-Jul(37.32)	19-Jul(29.88)	19-Jul(29.88)
D40	August	2000	29-Jul	01-Aug(41.14)	01-Aug(41.14)	31-Jul(47.85)	31-Jul(47.85)	01-Aug(39.35)	01-Aug(39.35)
D43	August	1400	12-Aug	13-Aug(70.77)	13-Aug(70.77)	13-Aug(78.25)	13-Aug(78.25)	13-Aug(63.93)	13-Aug(63.93)
D43	August	2000	12-Aug	14-Aug(64.81)	14-Aug(64.81)	13-Aug(74.04)	13-Aug(74.04)	14-Aug(57.25)	14-Aug(57.25)
D43	August	2400	12-Aug	16-Aug(22.45)	16-Aug(22.45)	16-Aug(28.54)	16-Aug(28.54)	16-Aug(22.70)	16-Aug(22.70)
D45	September	1400	28-Aug	30-Aug(73.22)	30-Aug(73.22)	29-Aug(81.44)	29-Aug(81.44)	30-Aug(66.63)	30-Aug(66.63)
D45	September	2000	28-Aug	31-Aug(44.84)	31-Aug(44.84)	31-Aug(55.61)	31-Aug(55.61)	31-Aug(45.91)	31-Aug(45.91)
D45	September	2400	28-Aug	31-Aug(59.67)	31-Aug(59.67)	30-Aug(72.93)	30-Aug(72.93)	31-Aug(53.37)	31-Aug(53.37)
D51	September	2000	18-Sep	21-Sep(44.75)	21-Sep(44.75)	20-Sep(56.37)	20-Sep(56.37)	21-Sep(46.91)	21-Sep(46.91)
D51	September	2400	18-Sep	22-Sep(36.45)	22-Sep(36.45)	21-Sep(46.45)	21-Sep(46.45)	22-Sep(38.39)	22-Sep(38.39)
D53	October	1400	2-Oct	04-Oct(70.97)	04-Oct(70.97)	03-Oct(78.47)	03-Oct(78.47)	04-Oct(64.11)	04-Oct(64.11)
D53	October	2400	2-Oct	07-Oct(40.75)	07-Oct(40.75)	07-Oct(45.06)	07-Oct(45.06)	07-Oct(36.82)	07-Oct(36.82)
D54	October	1400	2-Oct	04-Oct(59.86)	04-Oct(59.86)	03-Oct(76.29)	03-Oct(76.29)	04-Oct(63.05)	04-Oct(63.05)
D54	October	2000	2-Oct	06-Oct(45.74)	06-Oct(45.74)	05-Oct(58.29)	05-Oct(58.29)	06-Oct(48.17)	06-Oct(48.17)
D54	October	2400	2-Oct	07-Oct(38.53)	07-Oct(38.53)	06-Oct(47.71)	06-Oct(47.71)	07-Oct(38.79)	07-Oct(38.79)
D54	October	3000	2-Oct	08-Oct(38.60)	08-Oct(38.60)	08-Oct(43.73)	08-Oct(43.73)	08-Oct(35.02)	08-Oct(35.02)
D61	November	1400	6-Nov	10-Nov(55.11)	10-Nov(55.11)	10-Nov(62.83)	10-Nov(62.83)	11-Nov(50.30)	11-Nov(50.30)
D61	November	2000	6-Nov	12-Nov(41.04)	12-Nov(41.04)	12-Nov(49.99)	12-Nov(49.99)	13-Nov(40.57)	13-Nov(40.57)
D61	November	2400	6-Nov	13-Nov(46.01)	13-Nov(46.01)	12-Nov(51.17)	12-Nov(51.17)	14-Nov(41.86)	14-Nov(41.86)
D61	November	3000	6-Nov	19-Nov(28.29)	19-Nov(28.29)	17-Nov(35.09)	17-Nov(35.09)	19-Nov(28.97)	19-Nov(28.97)

Table C.7: Boatright and Tomberlin TOP date maximum estimation and percentage (%) ADH coverage by weight in 2013. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D51	September	1400	22-Sep	21-Sep(66.30)	21-Sep(64.49)	21-Sep(69.83)
D51	September	2000	22-Sep	26-Sep(59.12)	26-Sep(66.99)	26-Sep(53.65)
D51	September	2400	22-Sep	28-Sep(35.45)	28-Sep(43.89)	28-Sep(35.69)
D53	October	1400	10-Oct	15-Oct(79.48)	15-Oct(90.49)	15-Oct(72.61)
D53	October	2000	10-Oct	NA	NA	13-Oct(93.71)
D53	October	2400	10-Oct	15-Oct(37.61)	14-Oct(47.94)	15-Oct(39.62)
D53	October	3000	10-Oct	21-Oct(36.04)	19-Oct(40.63)	21-Oct(33.07)
D54	October	2000	11-Oct	14-Oct(69.58)	14-Oct(79.50)	14-Oct(61.46)
D54	October	2400	11-Oct	17-Oct(52.16)	15-Oct(57.55)	17-Oct(46.26)
D54	October	3000	11-Oct	22-Oct(28.41)	21-Oct(36.21)	22-Oct(29.92)

Table C.8: Boatright and Tomberlin TOP date maximum estimation and percentage (%) ADH coverage by weight in 2016. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D06	March	2000	19-Feb	29-Feb(38.78)	28-Feb(44.21)	29-Feb(35.40)
D06	March	2400	19-Feb	01-Mar(33.63)	01-Mar(39.11)	01-Mar(32.16)
D08	March	1400	29-Feb	02-Mar(57.89)	01-Mar(67.32)	02-Mar(55.37)
D08	March	2000	29-Feb	06-Mar(42.31)	05-Mar(49.20)	06-Mar(40.46)
D08	March	2400	29-Feb	09-Mar(45.89)	08-Mar(51.03)	09-Mar(41.76)
D23	June	1400	29-Feb	10-Mar(40.36)	09-Mar(44.53)	10-Mar(35.79)
D23	June	2000	16-Jun	NA	NA	NA
D23	June	2400	16-Jun	18-Jun(65.82)	18-Jun(72.62)	18-Jun(58.37)
D23	June	3000	16-Jun	20-Jun(49.00)	20-Jun(55.24)	20-Jun(44.96)
D26	June	1400	16-Jun	21-Jun(25.93)	21-Jun(30.16)	21-Jun(24.81)
D26	June	2000	21-Jun	23-Jun(86.83)	22-Jun(98.39)	23-Jun(78.79)
D26	June	2400	21-Jun	26-Jun(42.23)	26-Jun(52.38)	26-Jun(43.24)
D26	June	3000	21-Jun	27-Jun(34.08)	27-Jun(42.19)	27-Jun(34.31)
D28	June	1400	21-Jun	27-Jun(36.25)	26-Jun(40.08)	27-Jun(32.74)
D30	June	1400	21-Jun	16-Jun(63.76)	15-Jun(79.08)	15-Jun(65.29)
D67	February	1400	30-Jun	02-Jul(80.35)	02-Jul(88.84)	02-Jul(72.59)
D67	February	2000	26-Jan	07-Feb(58.66)	02-Feb(72.75)	07-Feb(60.06)
D67	February	2400	26-Jan	09-Feb(61.14)	03-Feb(67.46)	08-Feb(54.23)
D67	February	2400	26-Jan	NA	NA	10-Feb(77.67)

Table C.9: Boatright and Tomberlin TOP date maximum estimation and percentage (%) ADH coverage by weight in 2015. Grey cell indicates correct TOP prediction.

BodyID	Month	Milestone	Actual TOP	Pork/Equine 20.8° C	Pork/Equine 24.3° C	Pork/Equine 28.2° C
D23	June	1400	8-Jun	10-Jun(74.47)	10-Jun(84.78)	11-Jun(68.03)
D23	June	2000	8-Jun	12-Jun(52.24)	12-Jun(57.76)	12-Jun(47.19)
D23	June	2400	8-Jun	12-Jun(46.74)	12-Jun(52.96)	12-Jun(42.41)
D23	June	3000	8-Jun	14-Jun(54.69)	13-Jun(38.59)	14-Jun(31.57)
D24	June	1400	8-Jun	10-Jun(54.98)	10-Jun(68.20)	10-Jun(56.30)
D24	June	2000	8-Jun	13-Jun(54.39)	13-Jun(61.33)	13-Jun(49.92)
D24	June	2400	8-Jun	12-Jun(35.76)	12-Jun(45.57)	12-Jun(37.66)
D24	June	3000	8-Jun	14-Jun(35.04)	13-Jun(39.51)	14-Jun(32.16)
D25	June	1400	8-Jun	10-Jun(72.38)	09-Jun(82.02)	10-Jun(65.68)
D25	June	2000	8-Jun	11-Jun(62.34)	10-Jun(68.78)	11-Jun(55.29)
D25	June	2400	8-Jun	NA	11-Jun(78.71)	12-Jun(54.23)
D27	June	1400	5-Jun	NA	NA	07-Jun(94.08)
D27	June	2000	5-Jun	08-Jun(54.94)	08-Jun(61.95)	08-Jun(50.42)
D27	June	2400	5-Jun	09-Jun(46.98)	09-Jun(53.23)	09-Jun(42.63)
D27	June	3000	5-Jun	10-Jun(41.62)	10-Jun(45.92)	10-Jun(36.91)
D28	June	1400	4-Jun	08-Jun(55.99)	08-Jun(69.32)	08-Jun(56.37)
D28	June	2000	4-Jun	09-Jun(42.72)	09-Jun(49.68)	09-Jun(40.86)
D28	June	2400	4-Jun	08-Jun(36.63)	08-Jun(46.68)	09-Jun(38.58)
D30	June	1400	8-Jun	10-Jun(69.44)	10-Jun(76.78)	10-Jun(62.73)
D30	June	2000	8-Jun	12-Jun(54.73)	11-Jun(60.87)	12-Jun(49.80)
D30	June	2400	8-Jun	13-Jun(49.63)	13-Jun(56.51)	13-Jun(45.34)
D35	July	1400	2-Jul	04-Jul(60.88)	04-Jul(70.80)	04-Jul(58.23)
D35	July	2000	2-Jul	06-Jul(40.24)	05-Jul(49.02)	06-Jul(39.78)
D35	July	2400	2-Jul	07-Jul(33.00)	06-Jul(40.20)	07-Jul(32.63)
D35	July	3000	2-Jul	08-Jul(26.43)	07-Jul(30.14)	08-Jul(24.13)
D39	July	1400	15-Jul	17-Jul(93.47)	17-Jul(106.79)	17-Jul(82.57)
D39	July	2000	15-Jul	18-Jul(54.09)	18-Jul(59.80)	18-Jul(48.86)
D39	July	2400	15-Jul	19-Jul(35.92)	19-Jul(44.55)	20-Jul(36.78)
D40	August	2000	29-Jul	01-Aug(43.92)	01-Aug(54.48)	01-Aug(44.97)
D43	August	1400	12-Aug	13-Aug(73.69)	13-Aug(83.08)	13-Aug(67.62)
D43	August	2000	12-Aug	NA	14-Aug(92.04)	14-Aug(63.41)
D43	August	2400	12-Aug	17-Aug(34.58)	17-Aug(42.81)	17-Aug(34.81)
D45	September	1400	28-Aug	30-Aug(90.79)	30-Aug(80.20)	30-Aug(80.20)
D45	September	2000	28-Aug	01-Sep(57.33)	31-Aug(64.63)	01-Sep(52.61)
D45	September	2400	28-Aug	NA	NA	NA
D51	September	2000	18-Sep	21-Sep(55.12)	21-Sep(50.59)	21-Sep(50.59)
D51	September	2400	18-Sep	22-Sep(45.56)	22-Sep(50.67)	22-Sep(41.46)
D53	October	1400	2-Oct	04-Oct(76.09)	04-Oct(86.22)	04-Oct(69.05)
D53	October	2400	2-Oct	08-Oct(43.70)	07-Oct(49.51)	08-Oct(39.65)
D54	October	1400	2-Oct	04-Oct(75.57)	04-Oct(85.20)	05-Oct(69.35)
D54	October	2000	2-Oct	06-Oct(57.17)	05-Oct(63.59)	06-Oct(52.03)
D54	October	2400	2-Oct	07-Oct(41.03)	06-Oct(51.68)	07-Oct(43.01)
D54	October	3000	2-Oct	09-Oct(57.90)	08-Oct(58.97)	09-Oct(43.15)
D61	November	1400	6-Nov	14-Nov(61.23)	14-Nov(78.03)	14-Nov(64.49)
D61	November	2000	6-Nov	17-Nov(54.71)	17-Nov(60.85)	17-Nov(49.79)
D61	November	2400	6-Nov	15-Nov(56.13)	14-Nov(60.91)	15-Nov(48.07)
D61	November	3000	6-Nov	19-Nov(28.29)	17-Nov(35.09)	19-Nov(28.97)